REFLEX CONTROL OF ACTH, CORTISOL, AVP, AND RENIN RESPONSES TO SLOW HEMORRHAGE IN FETAL SHEEP

By

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To my parents Qinshan Chen and Xiuying He, and to my husband Ke Wu.

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KEY TO ABBREVIATIONS

ACTH	adrenal corticotropic hormone
ANOVA	analysis of variance
AVP	arginine vasopressin
BV_0	initial blood volume
BV_t	subsequent blood volume at each time point
CVP	central venous pressure
EDTA	ethylenediaminetetraacetic acid
FBS	fetal bovine serum
FRCV	the fractional red cell mass
Hct	hematocrit
Hct_o	initial hematocrits
Hct_t	subsequent Hct at each time point
HR	heart rate
MAP	mean arterial pressure
P_0	the plasma protein concentration before hemorrhage
$\%\Delta P_a$	actual % changes of plasma protein concentration
Pa_{CO2}	arterial P_{CO2}
Pa_{O2}	arterial P_{O2}
$\%\Delta P_c$	theoretical %changes plasma protein concentration
P_c	plasma protein concentration
pH_a	arterial pH
POMC	proopiomelanocortin

PRA	plasma renin activity
Prf	the protein concentration in restitution fluid
P_t	the protein concentration at each time point
PVN	paraventricular nucleus
RBC_0	initial red blood cell mass
RBC_t	subsequent red blood cell mass at each time point
%R	percent changes of the blood volume restitution
R_f	the blood volume restitution during hemorrhage
RIA	radioimmunoassay
SEM	standard error of the mean
SON	supraoptic nucleus
V_r	the blood volume removed during hemorrhage

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To investigate the afferent pathway for controlling fetal ACTH, AVP and PRA responses to slow hemorrhage, forty-six chronically catheterized late gestational fetal sheep were studied.

Experiment I investigated the role of the vagus nerve. One hundred and forty-three ml blood was withdrawn within two hours from five bilaterally vagotomized and six intact fetuses. No significant differences in the stimulation of the hormones were found between groups. The hormone stimulation was better correlated to the changes in pH_a than in blood pressures, suggesting that the responses in the fetus are possibly by chemoreceptors.

Experiment II tested the effect of chemoreceptors. HCl was infused at three different rates to fetuses. Infusions at the highest rate (0.50 meq/min) caused severe metabolic acidemia and significantly stimulated plasma ACTH and AVP but not PRA. It is speculated that the ACTH and AVP responses to acidemia are chemoreceptor mediated.

In Experiment III, 143 ml blood was withdrawn within two hours from twenty-six fetuses, which were either intact or bilaterally carotid sinus denervated plus vagotomized, in order to identify the peripheral chemoreceptors' effect during hemorrhage. Denervated

fetuses were studied with or without simultaneous infusion of phenylephrine. None of the hormonal responses was attenuated by denervation, and phenylephrine infusion attenuated or inhibited ACTH and AVP but not PRA when the exaggeration of MAP, pH_a and Pa_{CO2} in denervated fetuses was prevented. Thus the hormonal responses to slow hemorrhage was concluded not being mediated by peripheral chemoreceptors.

Experiment IV quantified fetal plasma ACTH and AVP responses to hypercapnia and tested the role of peripheral chemoreceptors. Six out of eleven fetuses were bilaterally carotid sinus denervated and vagotomized. Each subject was studied under normocapnia control and hypercapnia. Hypercapnia was induced by a CO_2 mixed-gas inhaled by the ewe. Hypercapnia doubled fetal plasma AVP; denervation did not attenuate the response. ACTH was not stimulated. We conclude that hypercapnia, associated with smaller decreases in pH_a than that in Experiment II, is not a stimulus to ACTH and is a mild stimulus to AVP. No hormone is controlled by peripheral chemoreceptors. The afferent pathway for mediating ACTH, AVP and PRA response to slow hemorrhage in fetal sheep needs to be investigated further.

CHAPTER 1
INTRODUCTION

Various intrauterine stresses, such as hemorrhage, hypotension, hypoxia, acidemia and hypercapnia, proved to stimulate ACTH, cortisol (Alexander et al., 1973; 1974b; Challis et al., 1989), AVP (Rurak, 1979; Drummond et al., 1977; 1978) and renin (Zubrow et al., 1988; Robillard et al., 1982) secretions in late gestational fetal sheep. Only a few experiments have been done to investigate the specific afferent pathways for mediating the hormonal responses to certain stresses. For example, the ACTH, AVP and renin responses to vena caval obstruction were significantly correlated to the magnitude of the decrease in blood pressure (Wood and Rudolph, 1983), and this response was mediated by afferent fibers in the carotid sinus and/or aortic nerves (Wood, 1989a). The findings that the response of vasopressin to hypoxemia is greater when the vagi are intact suggest a regulatory effect of the chemoreceptors (Rurak, 1978).

Hemorrhage has long been used as a stimulus for investigating the cardiovascular and endocrine responses to stress. In adult animals, the ACTH, cortisol and AVP (Wang et al., 1983) responses to hypotensive hemorrhage have been shown to be mediated by cardiovascular receptors located at the junction of the vena caval and atria, in the aortic arch, and in the carotid vasculature (Brennan et al., 1971; Cryer and Gann, 1973; Gann et al., 1964; Share and Levy, 1962; and Ludbrook, 1990). For mild or non-hypotensive hemorrhage, the mediator for the hormonal responses proved to be atrial type-B receptors (Cryer and Gann, 1973; Wang et al., 1983; Quail et al., 1987).

Fetuses have the ability to compensate more effectively for their volume depletion than adults. A slow hemorrhage, which usually causes little or no change in arterial blood

pressure, can initiate large hormonal responses. It is suggested that these hormonal responses are more important for fetal survival, because the fetal cardiovascular control mechanisms, such as autonomic innervation of the heart (which is the major and most rapid regulator of adult circulation), baroreflex and chemoreflex systems, etc., are not fully developed and fully functioning in some species, even at term. It is important to further investigate the regulation of hormonal responses to better understand the mechanism of fetal survival in intrauteral life, as well as the cardiovascular and endocrine developmental procedures.

Reflex control of the fetal hormone secretion depends upon the adequate development of related reflex components (sensors, afferent and efferent fibers and central nuclei) and the maturation of the related endocrine organs or cells. Based on adult studies, the circulatory reflex control system is very important to the hormonal responses mentioned above. We assume that this is also important in fetuses.

The fetal afferent pathway for regulating hormonal responses to slow hemorrhage remains unknown. The purpose of the studies in this dissertation is to investigate the afferent pathway regulating ACTH, cortisol, AVP and renin responses to slow hemorrhage in late gestational fetal sheep. Chapter 2 gives a background review of the developmental aspects of the circulatory control and the maturation of the related endocrine organs. Chapter 3 describes the general methodology of the experiments involved. Chapters 4 and 6 evaluate the effect of vagotomy (which interferes with the afferent fibers from atrial type B receptors), and vagotomy plus carotid sinus denervation (which denervates the peripheral baroreceptor and chemoreceptor afferent fibers) respectively, in fetal hormone responses to slow hemorrhage; chapters 5 and 7 study the chemoreceptors' effect on hormone responses to severe and milder hypercapnic acidemia, which was induced by HCl infusion in the former and maternal hypercapnic mix-gas inhalation in the latter. Chapter 8 is a summary and conclusion.

CHAPTER 2
BACKGROUND REVIEW

2.1 A Brief Review of the Fetal Circulatory System

The fetal cardiovascular system differs anatomically and functionally from the adult system to meet the growth and developmental requirements of intrauteral life. There are two circulations that are in parallel: the systemic and the umbilical–placental circulations. The umbilical vein and arteries are vessels that connect these two circulations. The ductus venosus carries most of the placental venous blood flow that bypasses the liver; the foramen ovale is an opening between the right and left atria located in the interatrial septum; and the ductus arteriosus is a vessel that connects the pulmonary trunk and the aorta. All these "designs" are based on the fact that fetal alveoli are fluid–filled; the gastrointestinal tract and the kidneys are much less functional than they become after birth. The two circulations ensure that the fetus gets enough oxygen and nutrients to supply it's growth and developmental requirement and to eliminate waste products.

Compared to the adult, the most remarkable features of fetal cardiovascular functions are the relatively higher cardiac output and heart rate (HR), which are 500 ml/kg/min and 150–180 bpm, respectively (Rudolph and Heymann, 1972; 1973), and lower mean arterial pressure (MAP), which is 36–44 mm Hg (Yardley et al., 1983) in late gestational sheep fetuses. The HR progressively falls and the blood pressure progressively increases in parallel with the growth and development of the fetus. In fetal lamb, the HR was reported to fall 0.67 beats/min/day and the MAP was rise 0.46 mm Hg/day from 100 days of gestation (0.68 term, term = 147 days) to term (Boddy et al., 1974b).

Fetuses have limited ability to increase their stroke volumes (Rudolph and Heymann, 1970; 1974; Rudolph et al., 1981). Examination of isolated fetal heart indicates that the heart muscle cells are smaller and the amount of noncontractile mass is considerably greater in fetuses than in the adults. Fetal sarcomeres are smaller in shape and are not well organized (Friedman, 1973). The fetal resting or passive tension of the heart muscle is higher and the compliance is much lower. The "stiffness" of the fetal heart restrict the performance of the heart. Although previous consumption that fetal heart behaves at the upper limits of its ventricular function curve (Gilbert, 1980) is proven to be incorrect because the influence of the stroke volume by after load was ignored (Hawkins et al., 1988), the ability of the increment of stroke volume in fetuses is still considered to be limited.

Almost half of the fetal cardiac output goes to the placenta through umbilical vessels. It is known that umbilical vasculature is not under neural control, but vascular resistance can be influenced by several vasoactive substances, such as angiotensin II, prostaglandins, bradykinin, etc. (Mott and Walker, 1983). Placenta flow is also influenced indirectly by systemic vascular tone. When systemic flow decreases or increases by vasoconstriction or vasodilation, the placental flow increases or decreases respectively. However, under normal physiological conditions, the fetus can always maintain an optimal flow ratio between these two circulations. The mechanism by which the fetus maintains the appropriate balance between placental and systemic circulations is not fully understood.

2.2 The Development of Autonomic Control of the Heart

The development of autonomic innervation of the fetal heart is the basis for the appearance of baro and chemoreflex control of the circulation. Reports from studies of rapidly developing chick embryos reveal that there are receptors and inactivating enzymes for both parasympathetic and sympathetic nerves on day 2 (0.1 term, term=21 days); cholinergic neuron growth within the sinoatrial node on day 6 (0.28 term); the first detectable release of

acetylcholine on day 10 (0.48 term), and the appearance of the functional vagal neurotransmission, acetylcholine, and the acetylcholine synthetic enzyme, choline acetyltransferase, on day 12 (0.57 term) (Pappano, 1977). Electrical stimulation of isolated human fetal atria also caused release of acetylcholine, and depressed contractility of the heart from 91 days of gestation (0.3 term). In fetal lambs, stimulation of the cervical vagus nerve produced a small inhibition of the heart as early as 60 days of gestation (0.4 term) (Dawes, 1968). There is also evidence that effective parasympathetic neurotransmission develops in guinea pig and rabbit fetuses (Vlk and Vincenzi, 1977). Myocardial innervation of the sympathetic nerve in most species is found to be incomplete at birth (Friedman et al., 1968; Lebowitz et al., 1972; Lipp and Rudolph, 1972). Sympathetic cardiac innervation in chick embryos was proved by histochemical technique, which followed the same sequence of cholinergic innervation, but the appearance of sympathetic nerves and the onset of effective neurotransmission was delayed (Pappano, 1977). A similar sequence of autonomic development occurs in man and other mammals, except the rat, which does not have cardiac sympathetic nerves until about the end of the first week of life (Marvin et al., 1980; Vlk, 1979). These species differences of the development of the autonomic nerve system are thought to correspond roughly to the general maturity of the animal at birth. The more dependent the species are on their mothers at birth, the less well developed the system is. The potential autonomic imbalance due to the delayed development of sympathetic nerves might be compensated for by the earlier development of cardiac adrenergic receptors. In sheep fetuses, β-adrenergic receptors can be stimulated at about 60 days of gestation (0.4 term) by appropriate agonists, which are ahead of the reported occurrence of sympathetic innervation (Barrett et al., 1972) and in parallel to the reported earliest stage of the parasympathetic stimulation (Dawes, 1968; Dawes et al., 1968). The point that greater development of parasympathetic than sympathetic nerve control of the heart, even at end of fetal life, has been very well proven and accepted.

2.3 The Development of Baroreflex Control of Circulation in Fetuses

Many efforts have been made to investigate the developmental aspects of arterial baroreflex control of fetal circulation and it has long been known that these receptors are active during fetal life. Two of the most common methods used in in vivo studies are: 1) demonstrating the reflex HR responses following changes in arterial and/or carotid sinus pressure, and 2) testing the HR response with or without intact baro-afferent nerve fibers. As early as the 1940s, Barcroft reported a functional baroreflex in anesthetized and exteriorized young fetuses in anesthetized ewes by injecting adrenaline and noradrenaline into lamb fetuses (Barcroft, 1946). He extended his findings to goat, rabbit and sheep fetuses by ligating their umbilical cords. The hypertension produced was associated with a significant bradycardia. He also proved that this HR response was abolished by vagotomy. Since then, numerous experiments in this area have been performed among different species.

It was found that the baroreflex was partially inhibited by carotid denervation and totally abolished by subsequent aortic denervation during fetal life (Rudolph and Heymann, 1973), which suggests that these two nerve fibers are important for the responses. Rudolph and his associates developed an unanesthetized, unstressed, and selectively peripheral baroreceptor denervated fetal model (Itskovitz and Rudolph, 1982). Since then, the function of these peripheral baroreflex afferent fibers has been better characterized. Baroreceptor activity was also demonstrated directly by recording the impulse from nerve fibers. Biscoe et al. (1969) measured phasic electrical activity in small bundles of fibers dissected from the carotid sinus nerve in 120-147 days of gestation (0.8-0.99 term) fetal sheep, and Ponte and Purves (1973) measured the activity in vago-afferent fibers in close-to-term sheep fetuses. They found that the measured nerve activity is synchronous with the arterial pulse, suggesting a functional baroreflex arc. Blanco et al. reported that spontaneous carotid and aortic

baroreceptor discharge is detectable from 85 days of gestation (0.6 term) in sheep fetuses (Blanco et al., 1985).

However, the earliest stage of the fetal lamb that can be proven to present a functional baroreflex control of the heart is 81 days of gestation (0.55 term) (MacDonald et al., 1980). The baroreflex control of the heart rate matures with advancing gestation. This is demonstrated by increased positive or negative HR responses to brief elevations or reduction of arterial pressure, respectively. Shinebourne et al. (1972) studied fetal lambs from 85–145 days of gestation (0.6–0.99 term) and found that baroreflex sensitivity progressively increased with advancing gestation until term. In younger fetuses, a sudden increase in aortic pressure was associated only sporadically with a baroreflex response. When it was elicited, the response was quite limited. Other studies also demonstrated that nearly all species experience a progressive postnatal maturation of the reflex to adult levels (Gootman et al. 1979; Vatner and Manders, 1979; Walker et al., 1990).

Further investigations tested the physiological role of baroreceptors in intrauterine cardiovascular regulation. The baroreflex was considered unimportant for fetuses except under conditions of elevated pressure, because the threshold of the reflex in adults is above the range of the normal fetal arterial blood pressure. This point was challenged by Yardley et al. (1979; 1983), who continuously recorded fetal arterial blood pressure during a 24-hour period in both intact and sino-aortic denervated fetal lambs. He found that denervation significantly increased the natural variability of arterial pressure. The coefficients of variations of mean arterial pressure (defined as stand deviation of the mean arterial pressure over the mean value of the mean arterial pressure) were twice as great in denervated fetuses compared to intact ones. This result is similar to the result from another experiment conducted by Cowley et al. (1973), who continuously recorded the BP within 24-hour on active, unanesthetized adult dogs. Marked fluctuations in fetal basal arterial blood pressure and heart rate after sinoaortic denervation were also observed by Itskovitiz et al. (1983),

although the MAP and HR are not different from control values. These results strongly suggest that arterial baroreceptors have a natural role in day-to-day fetal arterial pressure regulation.

Mechanoreceptors in atria, ventricles, and pulmonary arteries are potential sites of cardiovascular reflexes that have not been systematically investigated in the fetus. In 1975, Nuwayhid et al. (1975) reported that Bezold-Jarisch reflex could be stimulated by veritrodine injection in lamb fetuses close to term (Wt > 2700 g). In their experiments, the assumption that ventricular receptors affect fetal circulation was based on the reflex decrease of HR response to decrease of arterial pressure (Oberg, 1976). It is known that in adult animals, HR is accelerated by hemorrhage through arterial baroreceptor stimulation. In adult animals, only very rapid and severe hemorrhage can produce bradycardia, which is mediated by the increased firing rate of ventricular receptors that supersede the effect of arterial baroreceptors (Oberg and White, 1970). In fetuses, tachycardia only occurs when blood pressure is mildly reduced by hemorrhage or by venous occlusion (Wood et al., 1979; MacDonald et al., 1980). With greater decrease in pressure, the transient increase in heart rate is reversed and bradycardia occurs (Toubas et al., 1981; Wood and Rudolph, 1983a). Following atropine administration the bradycardia is abolished, indicating vagal efferent involvement of the reflex. These studies suggest that the ventricular receptor is functional during fetal life.

2.4 The Development of Chemoreflex Control of Circulation in Fetuses

In adult life, besides their dramatic effects on respiration, chemoreceptors are also important in the control of circulation. The peripheral chemoreceptors are located in the carotid and aortic bodies, and the central chemoreceptors are in the medulla oblongata. The stimulation of the central chemoreceptors alone always results in a reflex tachycardia and a very substantial increase in arterial blood pressure. Both effects are thought to be protective,

an attempt to circulate more blood through the affected areas in order to bring about a decrease in carbon dioxide tension or an increase in oxygen tension. Also, it has been suggested that the primary effect of carotid body stimulation in adult animals is bradycardia (De Burgh Daly, 1972), and by contrast, the effect of aortic chemoreceptor stimulation produces tachycardia (Sleight, 1974). The chemoreceptors of the aortic body in adults are considered to be more important than those of the carotid body in circulatory regulation (Comroe et al. 1964).

The aortic chemoreceptor was receptive to stimulation at about 100 days of gestation (0.68 term) (Dawes et al., 1969a; 1969b). An increase in arterial blood pressure, a decrease in HR, and vasoconstriction in the hind limb occured when arterial Pa_{O2} and Pa_{CO2} was changed over the physiological range, or when cyanide or nicotine was infused into fetal lambs. These responses can be abolished either by vagotomy or aortic denervation (Baillie et al., 1971; Dawes et al., 1969a; 1969b), which have specifically demonstrated the aortic chemoreceptor's effect. Direct recordings of fetal afferent electrical activity in aortic chemoreceptor fibers (Ponte and Purves, 1973) reveal that the responses to asphyxia and chemical stimulation are similar to those in the adults. The point that aortic chemoreceptors are active and important for fetal cardiovascular regulation has been very well accepted.

Early reports from fetal carotid chemoreceptor studies are controversial. Some claim that they are not functioning before birth, based on early direct nerve fiber recording studies and acute hypoxia studies (Cross and Malcolm, 1952; Dawes et al., 1982, Boddy et al., 1974; Dawes et al., 1969a; Purves and Biscoe, 1966), and some claimed that it is active based on response to infusion of cyanide (Dawes et al., 1969b). Others observed that carotid denervation showed no effect on fetal breathing movement, and only large quantities of chemical stimulation of the carotid body affect fetal breathing movement (Jansen et al., 1981; Purves, 1981). These results led to a conclusion that carotid chemoreceptor is inactive or plays only a small part, if any, in intrauteral fetal life regulation. However, later studies, most

from chronic animal preparations, suggested that the carotid body was more active prenatally than previously thought. Blanco et al. (1982) made direct recordings from carotid afferent fibers in fetal sheep and found that a random activity at about 5 Hz occurred at a Pa_{O2} of 25 mm Hg. The firing rate was increased by retrograde injection of CO_2-saturated saline into the lingual artery. Itskovitz and Rudolph (1987) performed carotid sinus denervation in sheep fetuses at 120 days of gestation (0.8 term). The cardiorespiratory response to intracarotid injection of cyanide was eliminated, which also supports the observation that carotid chemoreceptors are functional during at least the last third of gestation. A resetting of the chemoreceptor sensitivity on the day of birth and 5–10 days after birth was also reported (Blanco et al., 1984; Hanson et al., 1986). The carotid chemoreceptor is actually more active than previously thought.

Some reports indicate that direct stimulation of central chemoreceptors in fetal sheep increases fetal heart rate (Jansen, 1975; Jansen and Read, 1977; Jansen et al., 1981). However, most of the studies that test brainstem chemosensitivity of the brainstem in fetal lambs were related to the regulation of fetal breathing movement. Ventricular–cisternal perfusion with artificial cerebra-spinal fluid containing various concentrations of HCO_3^- increased the depth and incidence of breathing movements as CSF[H^+] increased from 45 to 73 neq/L (Bissonnette et al., 1980). A delayed fetal breathing movement was observed after intravenous infusion of NH_4^+ and HCl, which can be attributed to slow penetration of the H^+ into the interstitium of the brain (Hohimer and Bissonnette, 1981). The developmental aspect of central chemoreceptors and the interaction of the central and peripheral chemoreceptors during fetal life need to be further studied.

2.5 Fetal ACTH, Cortisol, AVP and Renin Responses

2.5.1 ACTH and Cortisol

The structural and functional development of the hypothalamus–pituitary–adrenal axis has been intensively investigated since 1960s. The glucocorticoids have been demonstrated to play a vital role in fetal life in various species. Glucocorticoids are involved in the maturation of several fetal organs or tissues, including the fetal lungs, adrenal medulla and small intestine. Glucocorticoids also participate in initiating parturition, and in mediating stress responses, which are important for fetal survival.

In human fetuses, the hypothalamus and pituitary are structurally showed to exist and pituitary hormones in granules can be detected at 42 days gestation (0.18 term); the hypothalamo–hypophysial portal system begins, and the releasing factors are identified at about 49 days gestation (0.2 term). By 70 to 91 days of gestation (0.29–0.37 term), the pituitary and hypothalamic tissues can respond in vitro to stimulatory or inhibitory stimuli. By mid–gestation, the fetal hypothalamic–pituitary axis is functional and the feedback control mechanism can be demonstrated. The pituitary–portal system presents as early as 49 to 56 days (0.2–0.23 term) and is complete at 126–140 days (0.53–0.58 term) in humans (Decherney and Naftolin, 1980).

The early development of the hypothalamus and pituitary is also established in other species, including sheep. Proopiomelanocortin (POMC), a precursor of ACTH and three other peptides: pro–γMSH, β–LPH and β–endorphrin, were detected by an immunocytochemical staining technique in the pituitary as early as 38 days of gestation (0.26 term) in sheep fetuses (Mulvogue et al., 1986) and at 34 days (0.3 term, term = 114 days) in the porcine pituitary (Dacheux, 1984). In the intermediate lobe of sheep fetuses, the POMC cells did not stain to show positively until 60 days of gestation (0.4 term). From 70 days on (0.48 term), the number of immunoreactive cells gradually increases, and by 90 days (0.61 term), almost all

the pars intermedia cells were intensely stained (Perry et al., 1985). Both "fetal" type corticotrophs (cells that are unique to the fetal pituitary) and "adult" type corticotrophs, which both contain POMC, were discovered in the fetal pituitary. Before 130 days (0.88 term), the "fetal" corticotrophs were predominant cell types; then these cells gradually disappeared and were replaced by the "adult" corticotrophs before term (Perry et al., 1985). By using ink-filling techniques, the hypothalamo-hypophysial blood system in sheep fetus was found to be patent from 45 days gestation (0.31 term), which gives evidence that the pituitary is potentially able to respond to hypothalamic releasing factors from this age onwards (Levidiotis et at., 1989). The fetal pituitary is responsive to AVP stimulation, which causes it to secrete substantial amounts of ACTH before the appearance of hypothalamic CRF. CRF terminals cannot be identified immunocytochemically in median eminence until around 100 days of gestation (0.68 term) (Levidiotis et al., 1987). AVP is present from 42 days (0.29 term), with the majority of AVP fiber terminations being found in the external zone of the median eminence. Norman and Challis (1987b) intravenously injected equimolar AVP and CRF into sheep fetuses on days 110–115, 125–130, 135–140 of gestation (0.75–0.78, 0.85–0.88, and 0.92–0.95 term, respectively). They found that the ovine fetal pituitary responds separately and synergistically to AVP and CRF on days 110–115 (0.75–0.78 term), but the role of AVP in stimulating ACTH release decreases with progressive gestational age. Plasma ACTH in sheep fetuses has been detected as early as 59 days gestation (0.4 term) (Alexander et al., 1973b), which also indicates the early functioning of the anterior pituitary gland.

The sheep fetal adrenal is morphologically recognizable by day 28 of gestation (0.19 term) (Ravault and Ortavant, 1977), while the zonation was not apparent until 60 days gestation (0.41 term) (MacIsaac et al., 1989; Webb, 1980). The sheep fetal adrenal gland, a highly active endocrine organ, is able to synthesize a considerable range of steroid hormones. There are adrenal structural and functional differences in sheep fetuses as compared to primates, including a permanent adrenal cortex instead of a fetal zone. This adult zone has

both 3-βHSD and Δ^5 isomerase activities; thus the fetuses do not need to rely on the placental source of progesterone for cortisol biosynthesis (Anderson et al., 1973). A slow, steady growth of the fetal adrenal was observed between 80 and 135 days (0.54 and 0.91 term), followed by explosive growth during the last 10–15 days of fetal life. This increase in weight is almost entirely due to an increase in the size of the adrenal cortex, and within the cortex the major growth occurs in the zona fasciculate (Comline and Silver, 1961; Liggins, 1969; Nathanielsz et al., 1972). When comparing with the weight of the adrenal gland relative to body size, the adrenals were maximal at an early stage of gestation (40–80 days, 0.27–0.54 term) in sheep fetuses (Wintour et al., 1975), 32 days (0.48 term, term=67 days) in guinea pigs (Moog and Ortiz, 1957), and 84–140 days (0.35–0.58 term) in humans (Lanman, 1953; 1962). Wintour et al. (1975) studied the adrenal function from 40 days gestation (0.27 term) in sheep fetuses. They found that the cortisol output per g body weight was greater in the fetal adrenal than the term adrenal when the adrenal glands were incubated with ACTH. The ACTH-stimulated effect on cortisol secretion declined significantly from 91 to 120 days gestation (0.62 to 0.82 term). Wintour et al. also measured peripheral blood level of cortisol in fetus between 60 days and term sheep fetuses (0.41 to 0.99 term), plasma cortisol concentration was significantly lower in 90–120 day fetuses (0.61–0.82 term) than in the younger or older ones. These findings suggest that the fetal adrenal becomes relatively quiescent and partially loses its sensitivity to ACTH after an early period of activity from 40 to 90 days gestation (0.27 to 0.61 term). This quiescent period is accompanied by a decrease in 17-hydroxylase and SCC mRNAs in the inner zone of the cortex (MacIsaac et al., 1989). Studies show that the plasma basal level of cortisol, accompanied by ACTH, starts to increase again 15–20 days before parturition (Hennessy, et al., 1982; MacIsaac et al., 1985; Magyar et al., 1980 and Norman et al., 1985). Some investigators divided the pattern of observed fetal plasma cortisol changes from 120 days (0.82 term) to term into four phases: phase 1 is the initial phase of low plasma cortisol concentration between 120–135 days (0.82–0.92 term) or so of gestation; phase 2 is 5-

13 days before delivery, which presents a slow initial increment of cortisol; phase 3 is a more rapid increase that occurs between 5 days before delivery and in the last 12–24 hour of fetal life; phase 4 is a very variable, final, stress–induced increment immediately before delivery (Nathanielsz, 1976).

The dependence of the growth of fetal adrenal on pituitary factor, most likely ACTH, was indicated by several studies. Hypoplasia of the fetal adrenal cortex (mainly in the zona fasciculate) and a fall in fetal plasma corticosteroid concentration were observed after electrocoagulation of the fetal sheep pituitary at 93 days gestation (0.63 term) (Liggins et al., 1966; 1968; Nathanielsz et al., 1972). Infusion of ACTH into the fetus reversed the deficiencies caused by hypophysectomy. The feedback regulation of cortisol on ACTH has also been shown by infusion of cortisol into fetuses, which causes inhibition of ACTH response to a variety of stimuli (Norman and Challis, 1987; Rose et al., 1985; Wood and Rudolph, 1983b). Bilateral adrenalectomy and therefore removal of cortisol feedback leads to an increase in fetal ACTH values after 125 days (0.85 term) (Wintour et al., 1980).

In terms of initiation of parturition, the fetal hypothalamo–hypophysial–adrenal axis plays a major role in sheep. Experiments that interrupted the normal connection between the fetal hypothalamus and pituitary by inserting a silicone plate between them, or by surgical ablation of the fetal pituitary or adrenal, resulted in the prolongation of pregnancy with failure of parturition to commence. Infusing ACTH or cortisol into the fetuses induced premature delivery (Challis and Olson, 1988; Liggins, 1969; Liggins et al., 1973; MacDonald and Porter, 1983; Thorburn and Charllis, 1979).

When the fetal hypothalamus–pituitary–adrenal axis response to stress are assessed from the increase of plasma ACTH and cortisol concentrations, the other sources of the hormone should be considered, including the placenta and the transplacental exchange. It is known that ACTH can be produced by the placenta but the physiological role of placental ACTH is uncertain. Although no change has been found in placental ACTH content as a

function of gestational age, placental ACTH can be stimulated under certain stress situations. Jones et al. (1988) have suggested that CRF may be released from the ovine placenta in response to the reduction of placental perfusion that results from β-agonist administration. Evidence from in vitro studies also indicate that CRF and ACTH are localized in the placenta, endometrium, and amnion (Challis JRG and Brooks AN, 1989). Carr et al. (1981) observed that the concentration of ACTH in maternal plasma rose progressively during gestation, independently of maternal glucocorticoids. This increase in ACTH might be from the placenta, which lacks an effect on maternal adrenal.

The placenta is impermeable to peptides, including ACTH, AVP, renin, angiotensin I and II, but is permeable to steroids, including cortisol. In another study, a five-hour intravenous infusion of cortisol into pregnant ewes raised the maternal plasma cortisol from 12.2 ± 4.2 to 44.0 ± 7.0 ng/ml and the fetal plasma values increased from 3.9 ± 1.5 to 12.0 ± 4.5 ng/ml (Wood and Rudolph, 1984), suggesting a significant transplacental movement of cortisol from mother to the fetus. A report from Dixon et al. (1970) also supports this conclusion. However, these ability of cortisol to move transplacentally seems limited, because of the presence of the diffusion resistance of the placenta to cortisol (Bietins et al., 1970; Liggins et al., 1973). This diffusion resistance establishes a constant plasma cortisol concentration gradient between ewes and their fetuses. Liggins et al. (1973) reported the resting gradient is about 5 fold greater concentration in ewe plasma than in fetal plasma (14 and 3 ng/ml in the ewe and fetus, respectively) by using isotope dilution techniques. Bietins et al. (1970) calculated the transplacental movement of cortisol from fetus to ewe and showed that the total contribution to the mothers by fetal plasma levels of cortisol is small. Since there is no evidence to show that the cortisol transplacental movement is unidirectional, we assume that cortisol can diffuse to the mother if the fetal cortisol concentration rises to a certain high level above the maternal concentration. These data suggest that the fetal plasma cortisol

concentration is determined by both fetal adrenal production and the transplacental diffusion movement, especially when the mother is under stress.

2.5.2 AVP

Vasopressin is synthesized in the supraoptic and paraventricular nuclei (SON and PVN) of the hypothalamus via a precursor protein, called propressophysin, which is packaged into neurosecretory vesicles within the neuronal perikaryon and enzymatically processed into vasopressin, neurophysin, and a C-terminal polypeptide during axonal transport. The early presence of vasopressin-generating cells can therefore be demonstrated by the precursor and the other two peptides in addition to vasopressin. The axonal terminals spread along the supraoptic-hypophyseal tract to the posterior pituitary, which is a storage terminal of the hormone. Axons containing neurohypophyseal hormones also project to the median eminence, where hormones can be secreted into portal vessels, and to other areas of the brain and spinal cord.

Both SON and PVN are formed between 12-14 days gestational (0.57-0.67 term, term = 21) in rats (Altman and Bayer, 1978a; Ifft et al., 1972) and mice (Shimada and Nakamura, 1973; Karim and Sloper, 1980). The neurons in these two species do not reach their mature size until the fourth week postnatally (Dellmann et al., 1981; Khachaturian and Sladekm 1980). A quantitative study on the maturation of the rat SON was tested by vasopressin-neurophysin antibodies (Khachaturian and Sladek 1980), showing that the neurophysin positive cells were minimal initially, then gradually increased to 27% at 17 days gestation (0.81 term) and reached 46% and 65% at 20 and 22 days gestation (0.95-0.99 term). The cell sizes also gradually enlarged to more adult-appearing cell bodies at end of gestation.

The ovine hypothalamo-neurohypophysial system is developed anatomically by 37-47 days gestation (0.25-0.32 term) (Diepen, 1941), and AVP is detected in the fetal lamb neurohypophysis at 55 days (0.37 term) (Rurak, 1979) and in plasma at 59 days (0.40 term)

(Drummond et al. 1980). Although AVP concentration in fetal sheep pituitaries was found to double between 90–143 days gestation (0.61–0.97 term) (Alexander et al., 1973b), the resting plasma AVP concentration is normally very low, close to or somewhat lower than adult levels (Alexander, 1974). It is unlikely that AVP is involved in normal circulatory regulation with such a low plasma concentration. Early data suggest that the clearance rate of AVP is higher in fetal lambs and rhesus monkeys than in adults (Allexander et al., 1976; Jones and Rurak, 1976; Robillard and Weitzman, 1980). The placenta was originally considered to be a major site of metabolism (Chard et al., 1973). However, when AVP metabolic clearance rates and production rates were measured in chronically catheterized fetal, newborn, and adult sheep by constant infusion, there was no significant difference among these three groups (Stegner et al., 1984). Wiriyathian et al. (1983) and DeVane et al. (1982) giving evidence that AVP was not cleared by the placenta.

It is known that in adult animals, AVP is important as an antidiuretic hormone, a potent vasoconstrictor, and an ACTH–releasing factor. AVP release is stimulated by rising blood osmolality or angiotensin II, by hypovolemia, and by a decrease in wall tension of the left atrium. The antidiuretic, vasoconstrictive and central ACTH–releasing effects of AVP have received much attention in developmental studies. It has been demonstrated in the past few years that AVP's antidiuretic and vasoconstrictor effects were mainly important in extreme situations during fetal life, such as during hemorrhage and hypoxia.

Hemorrhage is one of the most potent stimuli for AVP secretion. AVP can be stimulated by massive hemorrhage as early as 59 days gestation (0.40 term) (Drummond et al., 1980). A 10% reduction in blood volume in chronically catheterized fetal sheep aged between 96–124 days gestation (0.65–0.84 term) resulted in a rise in plasma AVP levels from 1.6 ± 0.23 to 9.6 ± 4.0 pg/ml, and a 20% loss produced levels of up to 31.7 ± 14.7 pg/ml (Drummond et al., 1980). Alexander (1971; 1974b) reported that the maximum AVP responses in fetal sheep can be stimulated by less than 40% of hemorrhage. Hypotension caused by nitroprusside infusion

without changes of blood volume also stimulates AVP secretion (Zubrow et al., 1988). Infusing the same amount of AVP as is produced by mild hemorrhage increased fetal blood pressure, decreased fetal heart rate, and redistributed blood flow in chronically catheterized fetal sheep (Iwamoto et al. 1979b). Kelly et al (1983) pretreated with vasopressin antagonist Δ-(CH_2)5Tyr(Me)AVP, found that 20% hemorrhage caused a significantly greater fall in BP than fetuses without an AVP antagonist. But AVP antagonist pretreatment did not change the basal fetal HR or MAP (Kelly et al., 1983). These results suggest that AVP is important for fetuses in the regulation of blood pressure following hemorrhage in fetuses. However, the unchanged basal HR and arterial pressure during AVP antagonist infusion suggest that AVP may not have much involvement in daily regulation of fetal circulation.

2.5.3 Renin

Renin, an enzyme that can convert angiotensinogen to angiotensin I, is also detected very early in fetal life. Renin has been extracted from the kidney of a 40-day gestational fetal lamb (0.27 term) (Wintour et al., 1977) and 22 days (0.19 term) from a pig fetus (Kaplan and Fridman, 1942). The lamb fetal plasma renin concentration was lower than maternal levels from 90 to 104 days gestation (0.61-0.70 term) (Carver and Mott, 1975), and progressively increased toward term. In the last quarter of gestation, the plasma renin concentration reached a level that is significantly higher in the fetal lambs than in their mothers (Broughton Pipkin and O'Brien, 1978; Broughton Pipkin et al., 1974a). Plasma renin activity (PRA), which is determined as the initial production rate of angiotensin I from angiotensinogen during incubation of plasma in vitro, was found consistently higher in chronically catheterized fetal lambs than in the ewes (Broughton-Pipkin and O'Brien, 1978; Broughton Pipkin et al., 1974a; Fleischman et al., 1975; Smith et al., 1974).

The high activity of the renin-angiotensin system in unstressed fetuses suggests that this system is important in regulating fetal circulation under physiological conditions. Infusion of Saralasin, a specific angiotensin receptor-blocking agent, into fetal lambs, resulted

in a fall in blood pressure, and the changes of blood pressure were linearly correlated to the initial angiotensin II level (Broughton-Pipkin and O'Brien, 1978). A rapid fall in fetal blood pressure was also observed when converting enzyme blocker captopril was administered to the mother. This fall in fetal blood pressure lasted for up to 3 days (Broughton-Pipkin et al., 1982). Hyman et al. (1975) found that chronic unilateral renal artery constriction in fetal lambs was associated with a marked elevation in both fetal plasma renin activity and arterial blood pressure. In addition to the effect on blood pressure, infusion of angiotensin II inhibitors to unstressed fetuses also decreased placenta flow (Iwamoto and Rudolph, 1982). Due to the immaturity of the fetal cardiovascular regulation system, it is possible that renin-angiotensin system plays a more important role in regulating circulation physiologically than it does in adults.

Hemorrhage in both anesthetized and unanesthetized fetal lambs, even as little as 3% of the calculated fetal blood volume, has been shown to stimulate PRA in fetuses from 110 days gestation to term (0.75 term) significantly (Broughton-Pipkin et al., 1974a; 1974b; Smith et al., 1974). In another study, a 12 ml hemorrhage stimulated PRA in 3 out of 5 anesthetized fetal piglets on 85 days of gestation (0.75 term). By 111 days (0.97 term), PRA increased in all eight piglets studied (Broughton Pipkin et al., 1981). Iwamoto and Rudolph (1981b) reported that previous administration of saralasin blocked the fetal lamb's ability to restore its blood pressure and heart rate following hemorrhage. These studies suggest that hemorrhage is as least as strong a stimulus for fetal renin secretion as it is in adults.

2.6 Summary

1. The fetal cardiovascular system differs anatomically and functionally from the adult to meet its growth and developmental requirements in intrauterine life.

2. The autonomic system starts to function at least before the end of the first half gestation, and the parasympathetic nerve system is more developed than the sympathetic nerve system at term.
3. Fetal baro and chemoreceptors undergo a development sequence and are active at the second half of gestation.
4. The ventricular receptors function in late gestational fetal sheep.
5. The fetal hypothalamus–pituitary–adrenal axis is active in the first trimester of fetal life. The adrenal becomes refractory to ACTH stimulation in the middle third of gestation, which is accompanied by a decrease in 17–hydroxylase and SCC mRNAs in the inner zone of the cortex. After this period, the adrenal becomes increasingly responsive to ACTH, and both ACTH and cortisol concentration in plasma increase shortly before term and reach their peaks at parturition.
6. The hypothalamo–neurohypophysial system appears to be anatomically developed by the first quarter of gestation. AVP acts as an early ACTH–releasing factor before and after the CRF is functional. AVP is important for responses to hypotension, but not for basal circulatory regulation in fetuses.
7. The renin–angiotensin system is important in regulating fetal circulation under both physiological and pathological conditions.
8. The placenta can produce ACTH and CRF and is permeable to corticosteroids but not to ACTH, AVP, and renin.
9. The fetuses respond to hemorrhage with an increase in ACTH, cortisol, AVP, and PRA. In adult animals, the afferent pathway for the hormone responses to mild– or non–hypotensive hemorrhage is mediated by atrial type–B receptors which is linked to vagus afferent fibers. The afferent pathway for fetal hormone secretion to slow hemorrhage remains unknown.

CHAPTER 3
GENERAL METHODOLOGY

3.1 Surgical Procedures of Catheterization and Denervation

All surgeries were performed at least four days before the experiments. For 24 hours before surgery ewes were not fed but were allowed free access to water. During surgery ewes were anesthetized with 1.0–2.5% halothane in oxygen. Using strictly aseptic techniques, we exposed the uterus with a midline incision. After incising the uterus, a fetal hind limb was delivered and a polyvinylchloride catheter (0.030 in. i.d., 0.05 in 0.d.) was inserted into the tibial artery. A larger catheter (0.040 in. id.,0.070 in. o.d.) was inserted into the saphenous vein. After catheterization of these two vessels, the fetal skin was sutured and the hind limb was returned to the amniotic cavity. Then the second fetal hind limb was delivered and the procedure was repeated. A polyvinylchloride catheter (0.050 in.i.d., 0.090 in.o.d.), with side-holes cut into the tip, was sutured to the skin before returning the second hind limb to the amniotic cavity. The amniotic catheter was used for measuring amniotic fluid pressure during experiments. The uterus was closed.

The surgical procedure for vagotomy and carotid sinus denervation was similar to that described by Itskovitz and Rudolph (1982). Briefly, after making another incision in the uterus, the fetus' head was delivered. A midline incision was performed in the fetus' neck near the angle of the jaw, the occipital–carotid arterial junctions were carefully exposed. The carotid sinus nerves, the IXth cranial nerves and the vagosympathetic trunks were in turn identified and sectioned as required. The superior thyroid arteries were ligated and cut, as were all branches of the common carotid arteries between the superior thyroid and lingual

arteries, to ensure sections of the common carotid nerves. The walls of the common carotid arteries in this area, as well as the lingual arteries and common carotid arteries extending 0.5–1 cm rostral from the lingual–carotid arterial junction, were stripped off all visible nerve fibers. The fetal skin was closed.

After closing the incision in the uterus, 500 mg ampicillin (Polyflex, Veterinary Products, Bristol Laboratories, Syracuse, New York) was injected into the amniotic fluid. Catheters were filled with heparin (1,000 units/ml; Wilkins–Sinn, Cherry Hill, New Jersey), plugged, and threaded out through a stab wound in the flank where they were protected by a cloth pouch sutured to the skin. In the case of twins, both fetuses were catheterized.

After the completion of the abdominal surgery, polyvinylchloride catheters (0.050 in.i.d., 0.090 in.o.d.) were inserted into the maternal femoral artery and vein at the level of the femoral triangle, and the tips were advanced to the abdominal aorta and inferior vena cava, respectively. The catheters also were filled with heparin, plugged, and routed subcutaneously to the flank, where they were protected by the same pocket as the fetal catheters. 500 mg ampicillin was injected intramuscularly into the mother at the time of surgery as well as once per day for five days after surgery. Fetuses were also treated with 500 mg ampicillin via the amniotic fluid catheter once every day for five days and after each experiment. All catheters were flushed and reheparinized at least once every 3 days.

3.2 General Preparation for Experiments and Hemodynamic Data Collection

The pregnant ewes used in all the experiments were mixed Western and Florida Native breeds. In the 2 series of hemorrhage studies, the animals participated in only one experiment. For those animals participating in more than one experiment in the other two studies (HCl infusion and hypercapnia), at least 48 hours were allowed between experiments, and the order of the experiments was randomized. No fetus was subjected to the same protocol more than once. All experiments were started between 0900 and 1100 hours to

prevent possible rhythm variations between animals in resting hormone concentrations or in the magnitude of stimulated responses.

On the morning of the experiment, the ewe to be studied was transported from the Health Center Animal Resources Department to the laboratory. At least one hour was allowed before the experiment started to let the ewe acclimate to the laboratory environment. During this time, one fetal femoral artery, vein, and the amniotic fluid catheter were connected to the transducers (Statham P23 Db or P23Id transducers, Statham Instruments, Oxnard, California) for measurement of intravascular and amniotic fluid pressures. Pressures were measured continuously using a Beckman R611 (Beckman Instruments, Schiller Park, Illinois) or Grass Model 7 (Grass Instruments, Quincy, Massachusetts) direct-writing recorder. Fetal heart rate was measured using an appropriate Beckman or Grass cardiotachometer triggered from the arterial pressure signal. Mean femoral arterial pressure was recorded as the damped mean of the phasic signal. Analog voltage outputs of the amplifiers were sampled by a Keithley DAS analog-to-digital converter (Keithley System 500, Keithley Data Acquisition Control, Cleveland, Ohio) and IBM AT microcomputer at a rate of 0.5 Hz. The variables were sampled and analog-to-digital conversions performed at 2-second intervals and stored on floppy disk. Intravascular pressures were corrected by subtraction of amniotic fluid pressure off-line. All fetal intravascular pressures were calculated using amniotic fluid pressure as zero reference.

3.3 Handling and Analysis of Blood Samples

All blood samples were placed in chilled plastic centrifuge tubes containing 0.05 ml of 0.3 M Na_4EDTA (Sigma Chemical Co, St. Louis, Missouri) per ml blood. All tubes were kept on ice until the end of the experiment, when they were centrifuged at 3,000 g for 20

minutes in a refrigerated (4°C) centrifuge. The plasma was stored at -20°C until hormones were assayed.

PRA was measured using a modification of the method by Haber et al., (1984). The kit was from Clinical Assays. For this assay, angiotensin I was generated in buffered (pH 5.7) plasma in vitro for one hour at 37°C. At the end of the incubation period the angiotensin I concentration was measured by RIA. This method has been described in detail elsewhere (Wood et al., 1989b).

Plasma cortisol concentrations were measured by RIA using antiserum No. 1460 (lot R2) from Radioassay Systems Laboratories (Carson, California) and [^{3}H]-[1,2,6,7]-cortisol from New England Nuclear Corp. (Boston, Massachusetts) or Amersham Co. (Arlington Heights, Illinois). Plasma was deproteinized with 50-100 vol of ethanol before assay.

Plasma ACTH was measured by radioimmunoassay (RIA) in either unextracted plasma (in the first two series of studies) or extracted plasma (in the last two series of studies, using powdered glass, eluted with HCl and acetone). The former used anti-ACTH antiserum and hACTH-(1-39) supplied by the National Hormone and Pituitary Program. The ^{125}I-ACTH used in this assay was made by iodination of the hACTH-(1-39) standard by the chloramine-T method (Rees et al, 1971). The latter used both anti-ACTH raised in this laboratory and 1-39 hACTH standard, which was supplied by the National Hormone and Pituitary Program (NIDDK, University of Maryland School of Medicine). This antiserum cross-reacts 100% with (1-24)-ACTH, 11-24 ACTH, and 1-39hACTH, but does not bind oCRF or methionine enkephalin.

Plasma vasopressin (AVP) concentrations were measured by RIA using anti-vasopressin antiserum purchased from Amersham, ^{125}I-vasopressin from New England Nuclear, and synthetic arginine vasopressin from Sigma.

In the first two series of experiments, plasma assayed for AVP was an extract of acetone as described by Cowley and co-workers (Cowley et al., 1981). In the last two series

studies, the plasma was extracted using bentonite, eluted with a 1:1 mixture of 1 M HCl and acetone. Extracts were dried using the Speedvac concentrator. The antiserum bound arginine vasopressin 0.5% relative to 1-39 hACTH.

Fetal blood gases were measured using a Radiometer PHM73 blood gas analyzer and BMS3Mk2 blood microsystem (Radiometer, Copenhagen, Denmark). The blood gas analyzer was calibrated as recommended by the manufacturer using two reference gas mixtures, one containing 8% CO_2, 21% O_2, and the balance N_2, and the other containing 5% CO_2 and the balance N_2 (Radiometer). Plasma protein concentration was measured by refractometry in study 3 (Chapter 6). Plasma sodium and potassium concentrations were measured using a NOVA 1 sodium/potassium analyzer (Nova Biomedical, Waltham MA).

3.4 Calculations and Statistical Analyses

The data were analyzed by two-way analysis of variance (ANOVA) corrected for repeated measures in one dimension, time (Winer, 1971). Changes were assessed within groups in some studies using one-way ANOVA for repeated measures. A posteriori comparison of individual means was performed using Duncan's multiple range test (Winer, 1971). Initial fetal and maternal variables were either tested by Student's t-test or by one-way ANOVA. In Experiment I (Chapter 4), correlations among variables were tested using standard correlations analysis. A significant level of $p \leq 0.05$ was used to reject the null hypothesis in all tests.

CHAPTER 4
THE ROLE OF VAGOSYMPATHETIC AFFERENT FIBERS IN THE CONTROL OF ACTH, VASOPRESSIN, AND RENIN RESPONSES TO HEMORRHAGE IN FETAL SHEEP

4.1 Introduction[1]

In adult animals, hemorrhage stimulates secretion of ACTH (Redgate, 1968), corticosteroids (Farrell et al., 1956; Gann, 1969), renin (Sapirstein et al., 1941), and AVP (Ginsburg and Heller, 1953) mediated via cardiopulmonary (Cryer and Gann, 1973; Brennan et al., 1971) and arterial mechanoreceptors (Redgate, 1968; Gann et al., 1964; Share and Levy, 1962). Renin is controlled by mechanoreceptors in those areas and by the intrarenal baroreceptor and the macula densa (Davis and Freeman, 1976). All three of these hormone systems also respond to hypoxia and/or hypercapnia, responses that may be at least partially mediated by the peripheral chemoreceptors (Raff et al., 1983a; 1983b; 1984). Results of experiments in adult animals suggest that atrial or other cardiac receptors with vagosympathetic afferent fibers are of primary importance in the control of ACTH, AVP, and renin responses to small or moderate hemorrhage (Cryer and Gann, 1973; Wang et al., 1983; Quail et al., 1987).

Fetal sheep respond to hemorrhage with increases in plasma ACTH (Alexander et al., 1973; 1974; Rose et al., 1978), AVP (Robillard et al., 1979; Rurak, 1979; Ross et al., 1986; Brace and Cheung, 1986), and renin (Robillard et al., 1982) concentrations. Presumably, receptor populations in the fetus that control the secretion of these hormones are similar to

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those in the adult. One might predict that, in the fetus as in the adult, the hormonal responses to a nonhypotensive or mildly hypotensive hemorrhage would be attenuated by interruption of the vagosympathetic afferent fibers from atrial or other cardiopulmonary receptors. This study was designed to investigate this possibility.

4.2 Materials and Methods

Ten chronically catheterized pregnant ewes and their fetuses were studied. One sheep carried twins; the others carried single fetuses. On the day of study, the fetuses were between 128 and 133 days' gestation.

Six fetal sheep (including the twins) were prepared with vascular catheters only. Five fetal sheep were subjected to bilateral section of the cervical vagosympathetic trunks.

Eleven ml blood samples were withdrawn from a fetal arterial catheter at 10-minute intervals for 120 minutes. At the beginning (0 minute) and end (120 minutes) of each experiment, 5 ml blood samples were drawn from the maternal arterial catheter. This hemorrhage paradigm is similar to that used by Brace and Cheung. Fetal blood gases were measured in all experiments.

4.3 Results

Initial fetal pH_a values were $7.39\pm.02$ and $7.39\pm.01$, Pa_{O2} 22.3 ± 1.4 and 24.0 ± 1.5 mm Hg, and Pa_{CO2} 43.3 ± 1.3 and 42.0 ± 1.1 mm Hg in the intact and vagotomized groups, respectively. None of these values were significantly different between groups.

Overall, hemorrhage decreased MAP (Figure 4-1, left). There was no significant difference in the MAP response to hemorrhage in the two groups. HR (Figure 4-1, right) was not significantly altered by the hemorrhage. Vagotomy did alter the CVP response to the

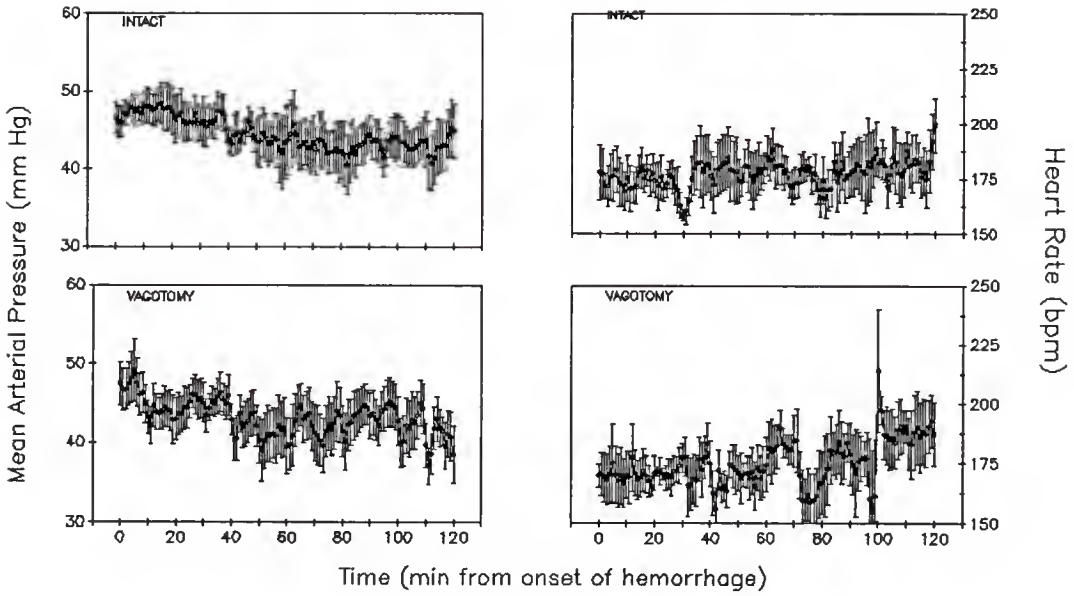

Figure 4–1. MAP and HR during hemorrhage in fetal sheep that were intact or subjected to bilateral section of cervical vagosympathetic trunks.

hemorrhage (Figure 4–2).

CVP significantly decreased in intact fetuses during hemorrhage. Hemorrhage significantly decreased fetal pH_a (Figure 4–3, bottom panel). The pH_a decreased more rapidly in the vagotomized group. In similar fashion, Pa_{CO2} (Figure 4–3, middle) increased significantly, but more rapidly in the vagotomized group. Pa_{O2} (Figure 4–3, top) decreased similarly in both groups equally.

Hematocrit (Figure 4–4) was significantly lower in the vagotomized fetal sheep at the beginning of hemorrhage (42±2% vs. 37±2% packed cell volume [PCV] in intact vs. vagotomized fetuses). The magnitude of the decrease in hematocrit during the course of the hemorrhage was greater in the intact fetuses (mean change, 6 % PCV) than in the vagotomized fetuses (mean change, 4 % PCV). Because the fetal sheep does not have a contractile spleen, acute changes in Hct have been used to calculate changes in blood volume (Brace, 1983). In the present experiments, the decrease in Hct during the hemorrhage may have been caused by transplacental and transcapillary refilling of the fetal vascular space. Red cells were diluted 14% in the intact group and 12% in the vagotomized fetuses, suggesting a more effective defense of blood volume in the intact group.

Initial fetal plasma ACTH concentrations were 41±8 and 60±7 pg/ml and initial fetal plasma cortisol concentrations were 9.1±3.3 and 7.8±2.2 ng/ml in the intact and vagotomized fetuses, respectively (Figure 4–5). Initial values of ACTH and cortisol were not different in the two groups. Hemorrhage stimulated similar ACTH and cortisol responses in both groups.

Initial fetal plasma AVP concentrations were 2.7±0.3 and 2.6±0.3 pg/ml and initial fetal PRAs were 5.0±1.5 and 4.7±2.2 ng angiotensin I/ml/hr (Figure 4–6). These initial values of vasopressin concentration and PRA were not different in the two groups. Hemorrhage stimulated similar AVP and similar PRA responses in the two group correlations of fetal plasma ACTH and AVP concentrations and PRA to fetal MAP, CVP, and pH_a were tested (Table 4–1). Logarithms of fetal hormone concentrations and activities were used in the

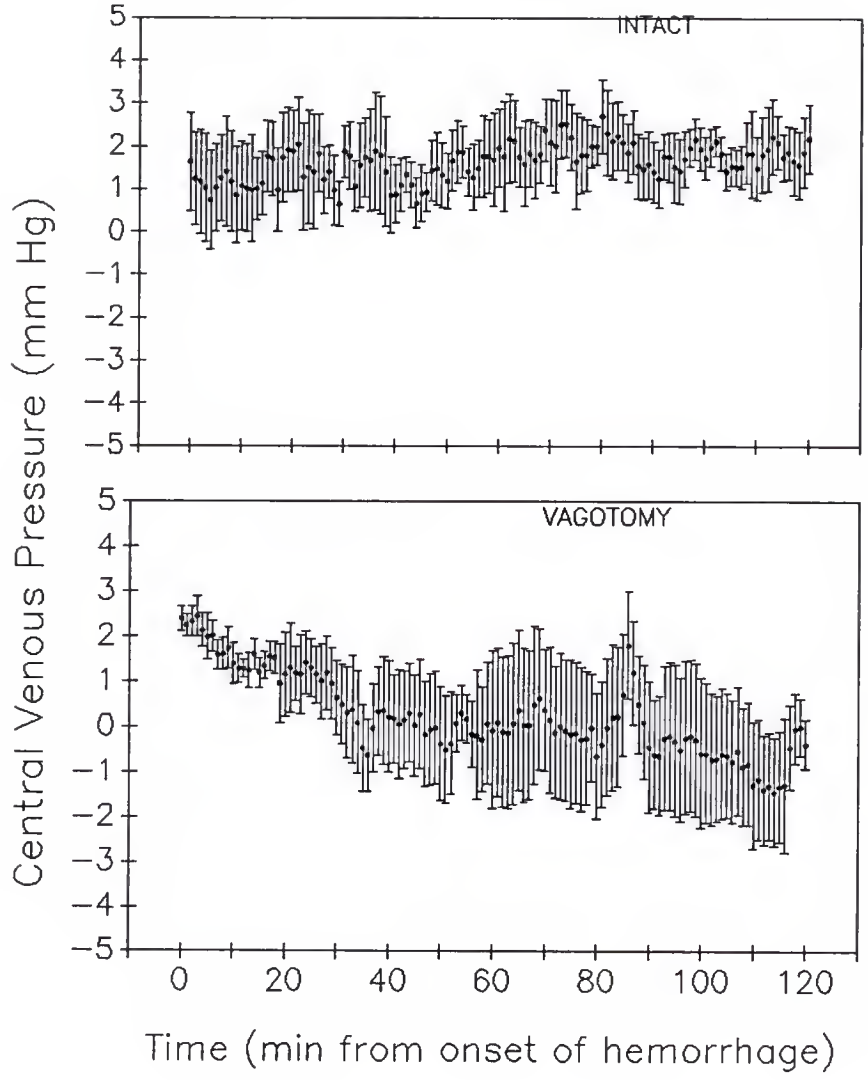

Figure 4-2. CVP during hemorrhage in fetal sheep that were intact or subjected to bilateral section of cervical vagosympathetic trunks.

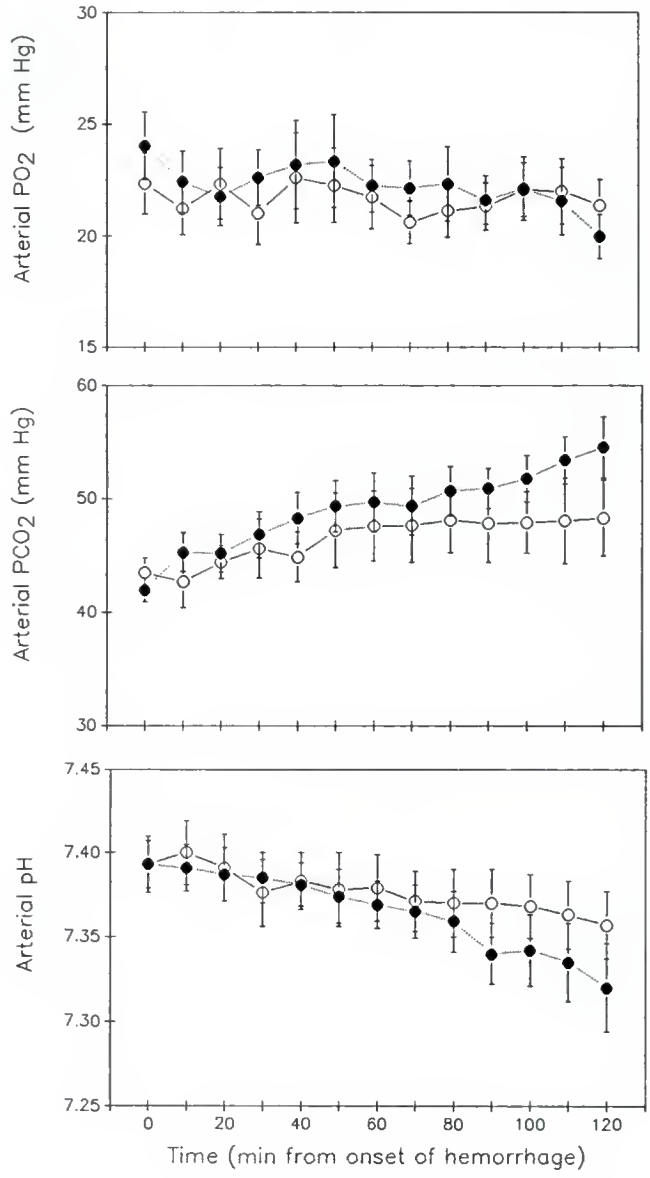

Figure 4–3. Pa_{O2}, Pa_{CO2}, and pH_a during hemorrhage in fetal sheep that were intact (open circles) or subjected to bilateral section of cervical vagosympathetic trunks (filled circles).

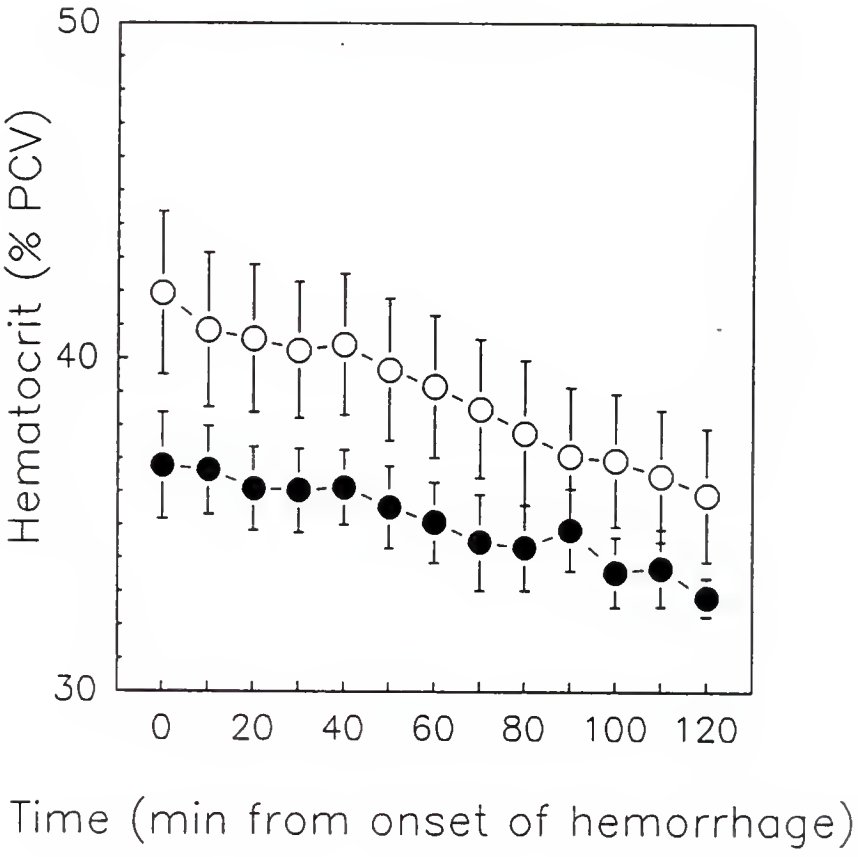

Figure 4–4. Hct during hemorrhage in fetal sheep that were intact (open circles) or subjected to bilateral section of cervical vagosympathetic trunks (filled circles).

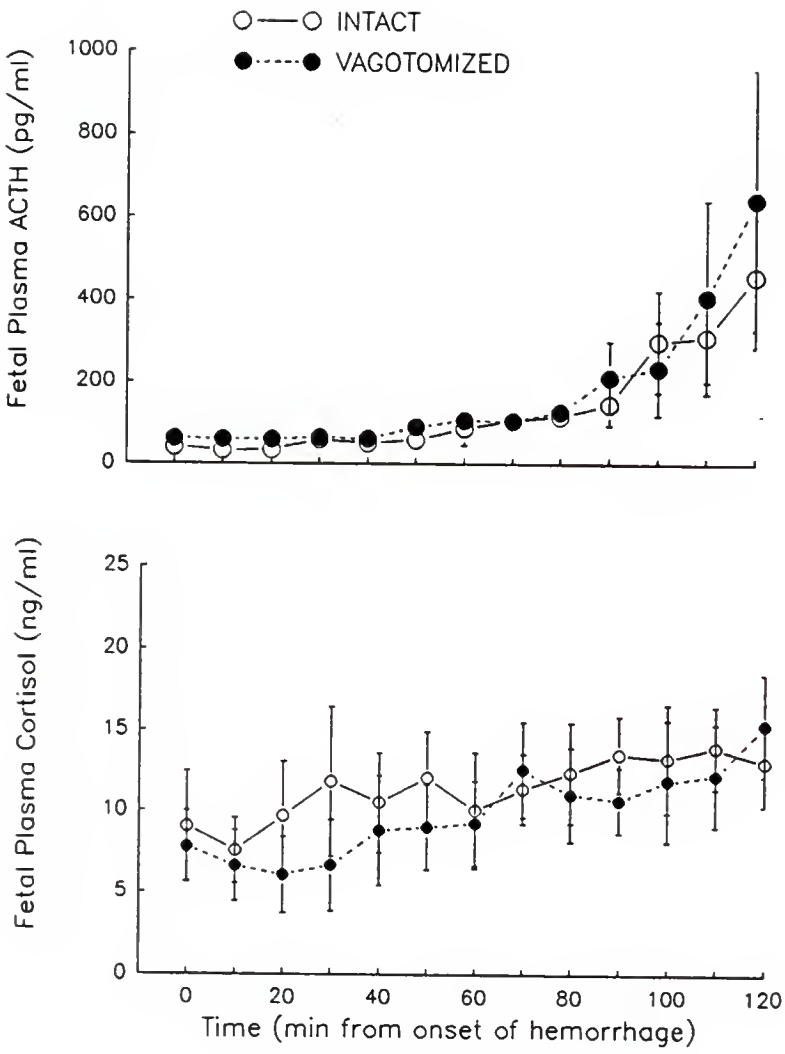

Figure 4–5. Plasma ACTH and cortisol concentrations in fetal sheep that were intact (open circles) or subjected to bilateral section of cervical vagosympathetic trunks (filled circles).

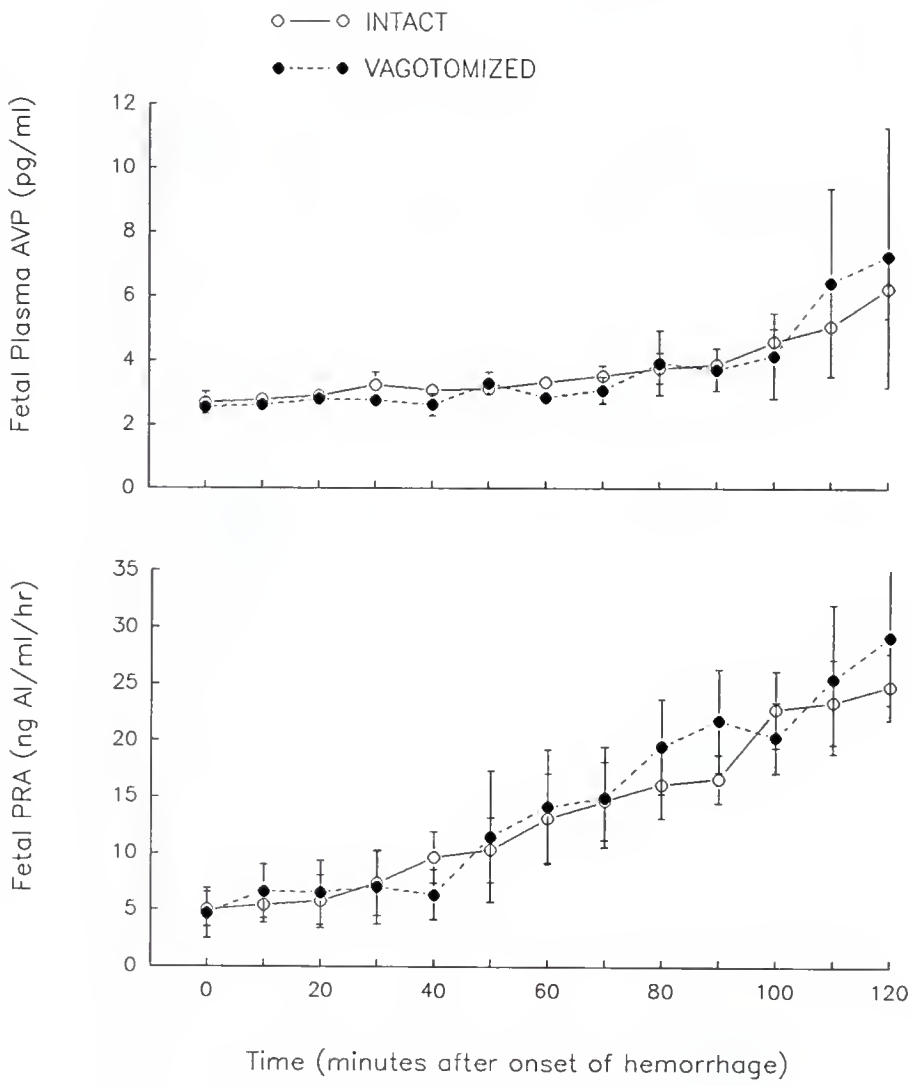

Figure 4-6. Plasma AVP concentration and PRA in fetal sheep that were intact (open circles) or subjected to bilateral section of cervical vagosympathetic trunks (filled circles).

Table 4–1. Values of correlation Coefficients Relating Fetal Plasma Hormone Concentrations to Arterial pH, MAP, and CVP.

	Log(ACTH) versus			Log(AVP) versus			Log(PRA) versus		
	pH	MAP	CVP	pH	MAP	CVP	pH	MAP	CVP
Intact Fetuses									
G17	−0.83*	0.39	−0.57*	−0.73*	0.44	−0.41	−0.72*	0.41	−0.53*
N407	−0.82*	0.36	−0.66*	−0.82*	−0.82*	0.02	−0.92*	0.33	−0.66*
OO74A	−0.93*	−0.23	0.02	−0.83*	0.07	0.23	−0.85*	−0.31	0.12
OO74B	−0.56*	a	a	−0.43	a	a	−0.54*	a	a
OO71	−0.64*	−0.15	0.52	−0.64*	−0.18	0.64	−0.44	−0.39	0.64
Y70	−0.28	−0.75*	0.74	b	b	b	−0.29	−0.72*	0.74
Vagotomized fetuses									
Y51	−0.99*	−0.69*	−0.66*	−0.91*	−0.73*	−0.73*	c	c	c
O247	−0.98*	−0.13	d	−0.98*	−0.10	d	−0.90*	−0.32*	d
Y63	−0.73*	−0.49	−0.19	−0.40	−0.36	−0.23	−0.84*	−0.40	0.16
Y64	−0.87*	−0.04	−0.30	0.55	0.26	0.61	−0.86*	−0.16	−0.60
Y236	−0.94*	−0.57*	−0.37	−0.83*	−0.47*	−0.34	−0.97*	−0.77*	−0.51

a, No hemodynamics in this experiment; b, no AVP measurements in this experiment; c: no PRA measurements in this experiment; d, no central venous pressure in this experiment.
* $p<0.05$.

correlations because logarithmic transformation linearized the relations (for example, see Wood et al., 1982). In one intact fetus, plasma ACTH was significantly related to MAP and CVP, and PRA was related to MAP. In one other intact fetus, vasopressin was significantly related to MAP. In one vagotomized fetus, ACTH and vasopressin were significantly related to both MAP and CVP, and in another vagotomized fetus, ACTH and PRA were significantly correlated to MAP (Table 4-1). Interestingly, in some of the experiments, ACTH, vasopressin, or PRA were correlated to increases in central venous pressure (e.g., fetuses Nos.0071, Y70, and Y64). In contrast to the inconsistent or seemingly inappropriate (increasing hormone concentrations with increasing intravascular pressures) correlations between hormone concentrations and arterial or venous pressures, the hormone responses were much better correlated to the changes in pH_a (Table 4-1). In 12 of 14 possible comparisons in intact fetuses and in 13 of 18 possible comparisons in vagotomized fetuses, correlations between hormone response and change in pH_a were significant. The mean values of fetal plasma ACTH, AVP, and PRA are graphically summarized in Figure 4-7. The relation between ACTH or vasopressin concentration or PRA and pH_a appeared to differ in the two groups (Figure 4-7). However, there was more variability in the slopes within groups than between groups. For this reason, there was no significant difference in the slopes between the two groups. Shown in Figure 4-8 are the distributions of correlation coefficients relating pH_a, MAP, and CVP to the logarithm of plasma ACTH, AVP, and PRA.

Maternal plasma hormone concentrations were measured at the beginning of each experiment. Maternal plasma ACTH concentrations were 102 ± 39 and 151 ± 14 pg/ml, and plasma cortisol concentrations were 12.3 ± 4.8 ns 16.1 ± 3.7 ng/ml in the groups with intact and vagotomized fetuses, respectively, Maternal plasma AVP concentrations were 2.5 ± 0.1 and 2.9 ± 0.3 pg/ml, and PRAs were 2.9 ± 0.7 and 2.4 ± 1.1 ng angiotensin I/ml/hr in the intact and vagotomy groups, respectively. There were no statistically significant differences in maternal plasma hormone levels in the two groups (tested by t test for independent groups).

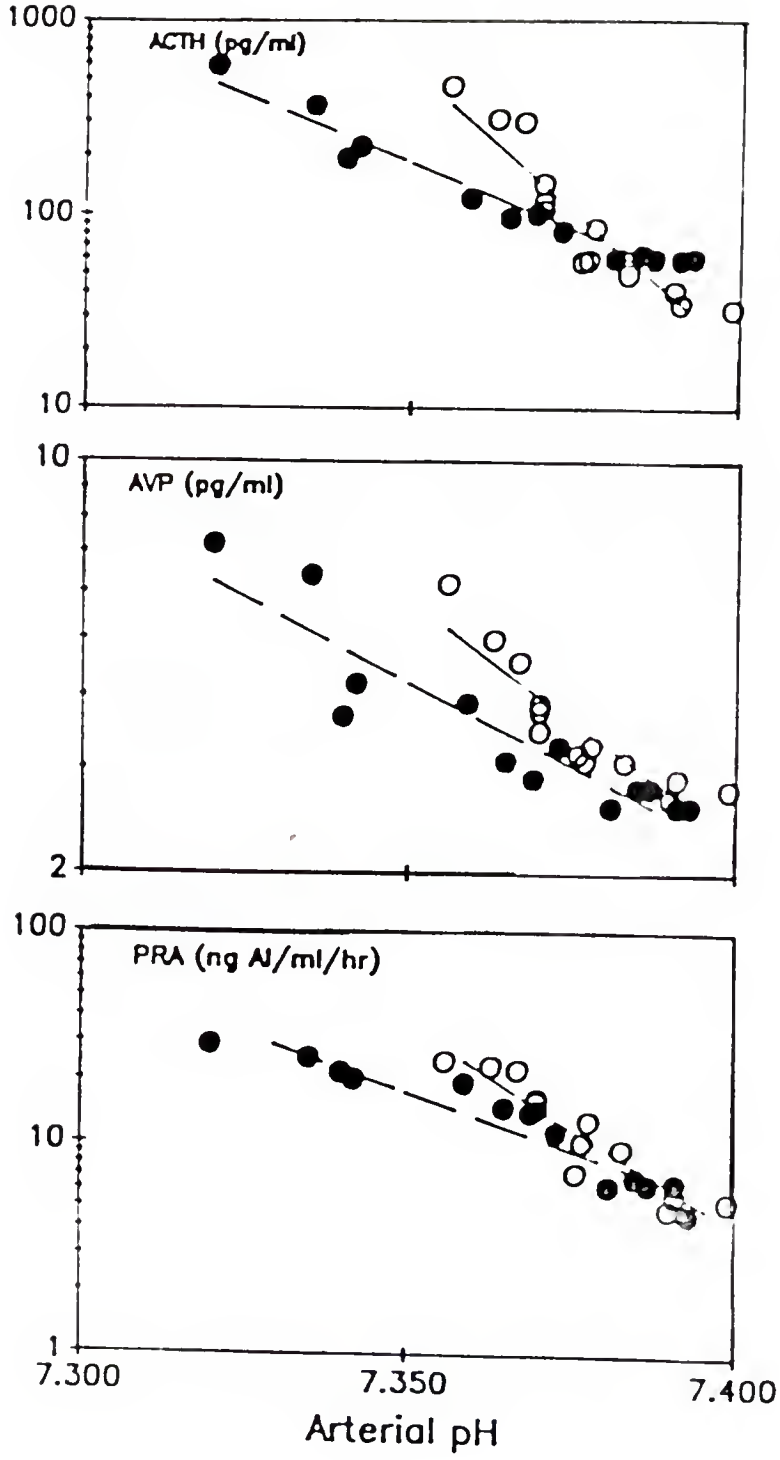

Figure 4-7. Relations among mean values of pH_a and mean values of fetal plasma ACTH and AVP concentrations and PRA.

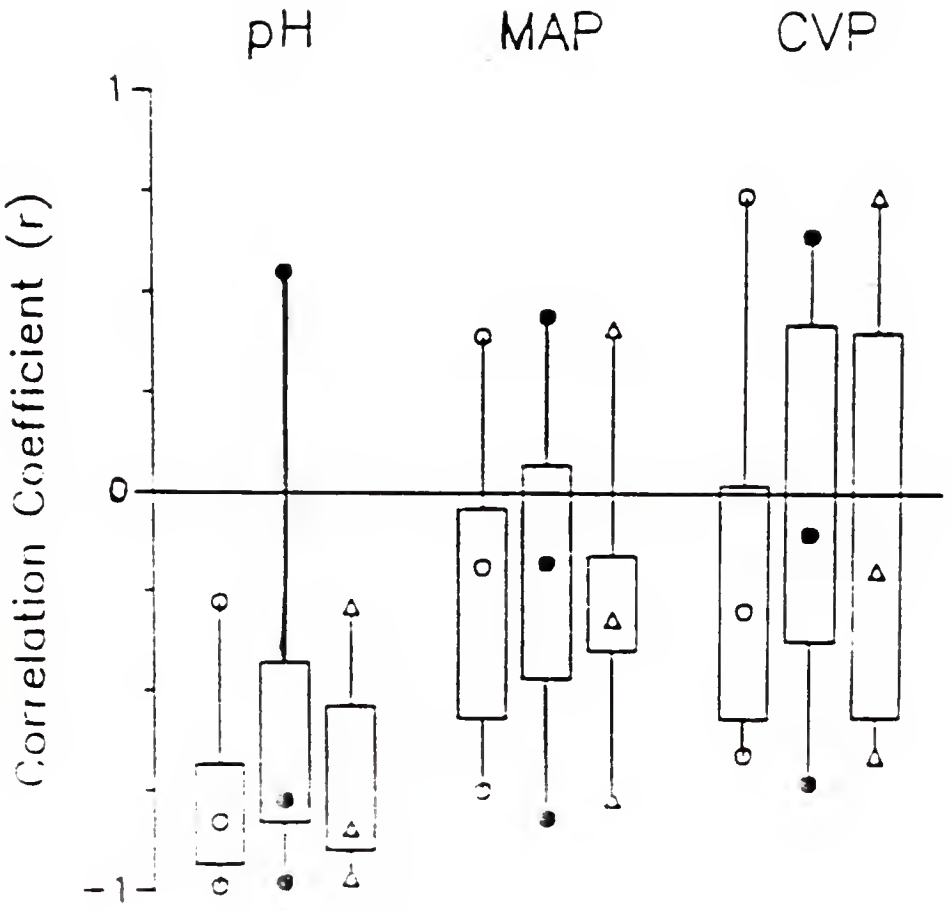

Figure 4-8. Distribution of r values of fetal pH_a, MAP, and CVP vs Lg ACTH (open circles), AVP (filled circles), or PRA (open triangle). Symboles at the extremes and inside box are the range of distribution and median values.

4.4 Discussion

The results of this study suggest that the control of hormonal responses to hemorrhage in fetal sheep is different from that in the adult. Section of the cervical vagosympathetic trunks, which interrupt afferent fibers from atrial and ventricular mechanoreceptors, did not alter the magnitudes of the ACTH, AVP or PRA responses to hemorrhage. In adult anesthetized dogs, acute vagotomy attenuated the vasopressin (Share, 1967) and adrenal corticosteroid (Gann and Cryer, 1973) responses to nonhypotensive or mildly hypotensive hemorrhage. ACTH and AVP in the adults are inhibited by afferent impulses from the atrial mechanoreceptors with afferent fibers in the vagus nerves (Brennan et al., 1971; Baertschi et al., 1976) and from carotid arterial mechanoreceptors (Gann et al., 1964; Share and Levy, 1962; Wood and Rudolph, 1983; Wood et al., 1985). Renin is controlled by these receptors and by the intrarenal baroreceptor (reviewed by Davis and Freeman, 1976).

The cardiopulmonary receptors are quite important in the mediation of the ACTH and AVP responses to slow or otherwise mild hemorrhage in adult animals. Renin responses to hemorrhage are less dependent on the activity of the cardiopulmonary receptors. Adrenal corticosteroid responses to 5 ml/kg hemorrhage in anesthetized dogs are attenuated by simultaneous inflation of a balloon in the right atrium (Cryer and Gann, 1973). Blockade of cardiopulmonary receptors with intrapericardial injections of procaine blocked the vasopressin but not the renin response to hemorrhage of a volume of up to 35% of blood volume in conscious rabbits (Quail et al, 1987). Similarly, cardiac denervation attenuated the vasopressin response but not the renin response to 10, 20, or 30 ml/kg hemorrhage in conscious dogs (Wang et al., 1983). While afferent information from arterial mechanoreceptors may contribute to the ACTH, AVP, and renin responses to hypotensive hemorrhage, the cardiopulmonary receptors appear to be most important. AVP and renin responses to

progressive hemorrhage to 35% of blood volume in conscious rabbits were not attenuated by sinoaortic denervation (Quail et al., 1987). ACTH and corticosteroid responses to hemorrhage in sinoaortic denervated lambs were not smaller than responses in intact lambs (Wood et al., 1985). Chemoreceptors may influence the secretion of ACTH, AVP, and renin in adult animals during hypoxia and/or hypercapnia (Raff et al., 1983a; 1983b; 1984), however, chemoreceptor stimulation is unlikely to mediate the responses of these hormones to small or moderate hemorrhage because carotid sinus denervation (which would eliminate afferent fibers from carotid arterial chemoreceptors and baroreceptors) does not attenuate the ACTH, AVP, or renin responses to hemorrhage in postnatal lambs (Wood et al., 1985).

Responses to hemorrhage in fetal animals differ from responses in adult animals in that moderate hemorrhage produces acidemia in addition to alterations in arterial and/or venous pressures (Toubas et al., 1981). The fetal acidemia is a respiratory acidemia caused by a primary increase in fetal Pa_{CO2}. This condition, analogous to hypoventilation by the adult, is most probably caused by decreased perfusion of the umbilical-placental circulation. Microsphere studies have demonstrated that hemorrhage reduces umbilical-placental perfusion (Itskovitz et al., 1982).

Fetal animals differ from adult animals in that MAP is regulated at a lower level. Chronic sinoaortic denervation in fetal sheep increased the variability of blood pressure and heart rate, indicating that fetal arterial baroreceptors are active at the regulated level of arterial pressure (Itskovitz et al., 1983). However, the firing rates of the fetal arterial baroreceptors may be at or near the threshold for activation. If this were true, fetal arterial baroreceptors would be less responsive to decreases than increases in fetal arterial pressure. Fetal arterial Pa_{O2} is also regulated at a somewhat lower level than that in the adult. It is known that fetal peripheral chemoreceptors are active at this low Pa_{O2} and that the sensitivity of these receptors to changes as arterial oxygen tension resets after birth to adult levels

(Blanco et al., 1984). Little is known about the characteristics of atrial receptors in fetal animals.

Several studies have demonstrated that fetal sheep respond to hemorrhage with increases in plasma ACTH and cortisol (Rose et al., 1978), AVP (Rurak, 1979), PRA, angiotensin II, and aldosterone (Robillard et al., 1982). It is known that the secretion of these hormones is influenced by induced changes in arterial or venous pressure. For example, ACTH and AVP are stimulated by vena caval obstruction in fetal sheep, and the magnitude of the responses is related to the severity of the obstruction (Wood et al., 1985), leading to the speculation that cardiovascular mechanoreceptors influence the secretion of these hormones. During hypovolemia in fetal sheep, plasma AVP and PRA responses correlated better to the volume of the hemorrhage than to the induced changes in MAP, leading to the speculation that volume receptors are important in the control of these two hormones (Robillard et al., 1979). A significant correlation between plasma AVP and pH_a during hypovolemia was noted in one study (Rurak, 1979). Other investigators have suggested that fetal hypoxia during hypovolemia may contribute to the vasopressin response (Ross et al., 1986). While several groups of investigators have studied hormonal responses to hemorrhage in the fetus, the populations of cardiovascular receptors responsible for mediation of these hormonal responses have received little attention.

As in studies by other investigators, the fetal hormonal responses to hemorrhage were not well correlated to the induced changes in arterial pressure (Robillard et al., 1982; Brace and Cheung, 1986). It is apparent from the results of the present study that fetal ACTH, AVP, and renin responses to mildly hypotensive hemorrhage in fetal sheep are more closely associated with the induced changes in arterial blood pH. The correlations with arterial and central venous blood pressure reveal mostly either nonsignificant relations or relations that are seemingly inappropriate for cardiovascular mechanoreceptor–hormone relations (increased hormone concentration with increased blood pressure). In contrast, the correlation

coefficients calculated from relations between hormone concentrations and pH_a are high in most experiments. The association of hormone response to changes in pH_a would therefore suggest the possibility that the responses of these hormones to mild hemorrhage in the fetal sheep are mediated by the peripheral chemoreceptors rather than mechanoreceptors. It is also possible however, that the hypercapnia produced by fetal hemorrhage stimulated the hormonal responses via medullary chemoreceptors. Raff and co-workers (Raff et al., 1984) demonstrated that deafferentation of the carotid chemoreceptors attenuated that ACTH and corticosteroid response to isocapnic hypoxia but had no effect on the responses to hypercapnic hypoxia in anesthetized adult dogs.

While vagotomy did not alter the final magnitudes of the fetal ACTH, AVP, or PRA responses to hemorrhage (Figure 4-5 and 4-6), it did alter the Pa_{CO2} and pH_a responses (Figure 4-3). The greater increases in Pa_{CO2} and decreases in pH_a in the vagotomized group may reflect a greater decrease in fetal cardiac output and a concomitant greater decrease in fetal umbilical-placental flow in the vagotomized fetuses.

Vagotomy also altered the CVP responses to the hemorrhage (Figure 4-2). Intact fetuses defended CVP well during this hemorrhage while vagotomized fetuses responded to the hemorrhage with a substantial decrease in CVP. The maintenance of CVP in the intact fetuses may have been dependent on rapid restitution of blood volume. Because fetal sheep do not release red blood cells into the circulation after acute sympathetic stimulation (Brace, 1983), changes in hematocrit can be used to follow acute changes in blood volume. Assuming the fetal body weight was approximately 3.3 kg (calculated from the relation of gestational age to fetal body weight) as described by Robillard and Weitzman (Robillard and Weitzman, 1980) and assuming that fetal blood volume is 110 ml/kg (Brace, 1983), we can estimate that approximately 33 ml (28% of the volume removed between the 0- and 120-minute samples) of fluid re-entered the fetal vascular space during the fetal hemorrhage. Using a similar approach, we can calculate that only 20 ml (17% of the volume removed) of fluid re-entered

the fetal vascular space in the vagotomized fetuses. This apparent difference in the rate of volume restitution may have been the cause of the greater decrease in CVP in the vagotomized fetuses. It is also possible that vascular compliance was lower in the vagotomized fetuses. In any event, the reduced rate of volume restitution and/or decreased vascular compliance may have contributed to an impairment of cardiac output and umbilical–placental blood flow, which, in turn, produced the greater increase in Pa_{CO2} and decrease in pH_a.

The difference in initial hematocrit in the intact and vagotomized groups may have also reflected alteration of fetal fluid balance by vagotomy. It is tempting to propose that the vagotomy produced an expanded plasma volume and, therefore, secondarily, a decrease in hematocrit by dilution of the red blood cells. This remains to be tested.

In summary, we have found that vagotomy altered the blood gas and CVP responses to slow hemorrhage in the sheep fetus but did not alter the magnitudes of the ACTH, AVP, or renin responses. The results suggest that the control of these hormonal responses to hemorrhage in the fetus is fundamentally different from that in the adult. The data suggest the possibility that hormonal responses to hemorrhage in the fetus are stimulated by chemoreceptor activity, secondary to the acidemia or hypercapnia of fetal hemorrhage.

CHAPTER 5
ACTH, AVP, AND RENIN RESPONSE TO INTRAVENOUS INFUSION OF HYDROCHLORIC ACID - ARE CHEMORECEPTORS RESPONSIBLE FOR FETAL HORMONE SECRETION?

5.1 Introduction[2]

The secretion of ACTH, AVP, and renin are stimulated by hemorrhage, hypotension, and hypoxia. In adult animals, the responses of these hormone systems to hemorrhage and hypotension are mediated by arterial and atrial mechanoreceptors (Brennan et al., 1971; Bunag et al., 1966; Cryer and Gann, 1973; Gann et al., 1964; Redgate, 1968; Share and Levy, 1962) and, for renin, by the intrarenal baroreceptor and the macula densa (Davis and Freman, 1976). Because resting mean arterial pressure is much lower in the fetus than in the adult, it is not clear that similar mechanisms govern the ACTH, AVP, and PRA responses to hemorrhage and hypotension in fetal sheep.

These endocrine systems were investigated in previous experiments to show their responses to vena caval obstruction (Wood, 1989a, Wood et al, 1982) and to slow hemorrhage (Chapter 4). Vena caval obstruction, a stimulus that produces a large and rapid decrease in fetal arterial pressure increased fetal ACTH, AVP, and PRA to levels that were related to the induced change in arterial blood pressure. Heart rate was decreased to levels significantly related to the induced change in arterial blood pressure. Sino-aortic denervation attenuated the ACTH, AVP, and heart rate responses, suggesting that the afferent pathways for these reflexes were interrupted by removal of either the mechanoreceptor or the chemoreceptor

[2] This work was in part supported by the National Heart, Lung, and Blood Institute grant HL-36289.

afferent fibers from the carotid sinuses or aortic arch (Wood, 1989a). The attenuation of the bradycardia normally observed during fetal hypotension suggested that these reflex responses may be mediated by chemoreceptor afferents rather than the mechanoreceptor afferents.

Slow fetal hemorrhage, a stimulus that produces small and prolonged decreases in fetal arterial pressure, increased fetal Pa_{CO2}, and decreased fetal pH_a; the changes in pH_a and Pa_{CO2} were probably secondary to reduced perfusion of the umbilical–placental circulation (Faber et al., 1973). The fetal ACTH, AVP, and renin responses to the hemorrhage were highly correlated to the change in pH_a but were not correlated to the induced change in fetal mean arterial or central venous pressure. We performed these experiments to test the hypothesis that the acute acidemia, in the absence of hypovolemia, stimulates fetal ACTH, AVP, and renin secretion.

5.2 Methods

Eight chronically catheterized pregnant ewes and their fetuses were studied. Two of these ewes carried twins; the other six carried singleton fetuses. On the days that the fetuses were studied, the ages ranged from 132 to 140 days gestation.

Hydrochloric acid (HCl) was infused into the fetal inferior vena cava at one of three different rates (0.02, 0.10, or 0.50 meq/min) for 60 minutes. All infusions were performed using a constant infusion pump (Sage Instruments model 341A, Cambridge, MA) at a volume rate of 0.5 ml/min, with HCl concentrations in the infusate adjusted to 0.04, 0.5, and 1.0 meq/ml. We performed five infusions of 0.02 meq/min, six infusions of 0.10 meq/min, and five infusions of 0.50/meq/min. Blood samples (5 ml/each) were drawn from a fetal femoral arterial catheter at the beginning of the infusion and thereafter at 10–min intervals throughout the infusion period. As each fetal blood sample was drawn, an additional blood sample (1 ml) was drawn into a heparinized syringe for measurement of fetal hematocrit and blood gases. Therefore a total of 42 ml was drawn from the fetal circulation during these experiments.

5.3 Results

Intravenous infusion of HCl at a rate of 0.5 meq/min significantly decreased fetal PH_a and increased Pa_{CO2} (Figure 5-1). Results of two-way ANOVAs are reported in Table 5-1. Pa_{O2} was significantly increased over all groups, but this increase was not significantly related to the rate of infusion. Infusion of HCl at a rate of 0.02 meq/min did not significantly alter fetal blood gases; however, infusions of 0.1 and 0.5 meq/min significantly decreased pH_a and increased Pa_{CO2} beginning 20 and 10 min after the onset of infusion, respectively.

Infusion of HCl did not significantly alter fetal arterial blood pressure at any of the rates tested (Figure 5-2, Table 5-1). Fetal heart rate was increased during infusion of 0.5 meq/min but not during infusion of 0.02 or 0.10 meq/min. Fetal heart rate was significantly increased over the initial level 54-61 min after the onset of infusion in the 0.5 meq/min group. Initial values of fetal blood pressure, and blood gases were not different among groups.

Fetal plasma ACTH and AVP were stimulated by infusion of HCl at a rate of 0.5 meq/min (Figure 5-3, Table 5-1). Fetal plasma cortisol concentration appeared to increase in all groups (Table 5-1 and 5-2). A posteriori comparison of individual means by Duncan's multiple range test indicated that in the 0.5 meq/min group, ACTH and AVP were significantly increased (relative to 0 min) at 30, 40, 50 and 60 min and that cortisol was significantly increased at 50 and 60 min (Table 5-2). Initial values of plasma ACTH and AVP concentrations were not different among groups). In Figure 5-4 we present the relationships between fetal arterial H^+ concentrations, Pa_{CO2} and the plasma concentrations of ACTH and AVP in the 0.5 meq/min group. Figure 5-4 shows that both hormones are increased at H^+ concentrations > 60 nM and Pa_{CO2} > 50 mm Hg. Because of the consistent relationship between blood H^+ and ACTH and AVP, there were significant correlations between pH_a and the logarithm of plasma ACTH ($r=0.843$, $n=35$) and AVP ($r=0.773$, $n=28$). The logarithms of

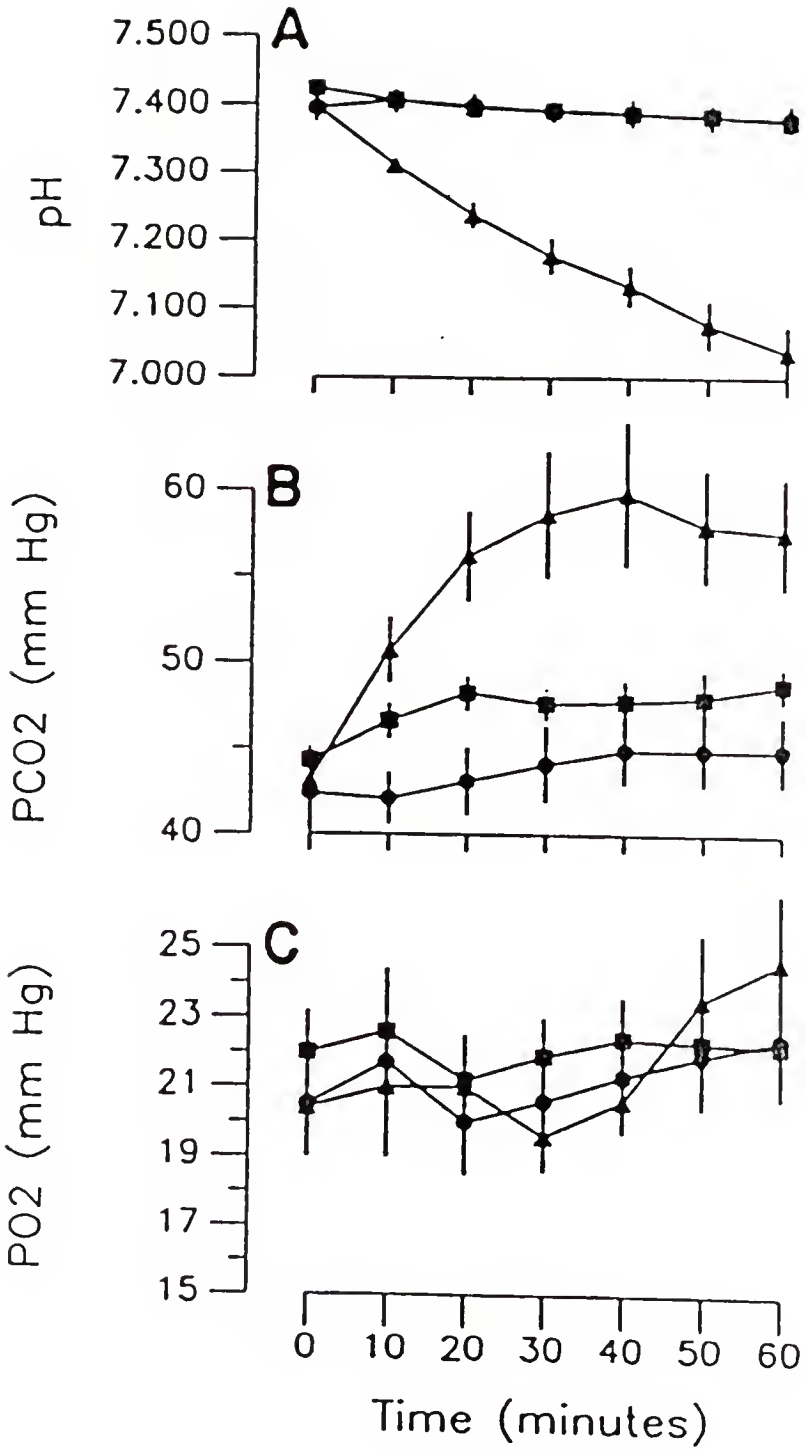

Figure 5–1. Mean fetal pH_a (A), Pa_{CO2} (B), and Pa_{O2} (C) during intravenous infusion of HCl at rates of 0.02 (filled circles), 0.10 (filled squares), and 0.50 (filled triangles) meq/min.

Table 5-1. Results of ANOVAs

Variable	F (df)		
	Dose	Time	Dose × time
pH	42.99* (2,13)	118.56* (6,78)	69.61* (12,78)
Pa_{CO_2}	8.00* (2,13)	36.04* (6,78)	12.65* (12,78)
Pa_{O_2}	0.13 (2,13)	5.40* (6,78)	1.78 (12,78)
Hct	1.48 (2,13)	6.81* (6,78)	1.32 (12,78)
Mean arterial pressure	0.87 (2,13)	1.35 (60,780)	1.00 (120,780)
Heart rate	0.12 (2,13)	3.95* (60,720)	1.63* (120,720)
ACTH	4.53* (2,13)	9.86* (6,78)	5.70* (12,78)
Cortisol	0.46 (2,12)	5.50* (6,72)	1.87 (12,72)
AVP	2.33 (2,10)	2.87* (6,60)	3.65* (12,60)
PRA	2.82 (2,13)	3.81* (6,78)	1.12 (12,78)

Pa_{CO_2} and Pa_{O_2}, arterial partial pressure of CO_2 and O_2, respectively; Hct, hematocrit; AVP, arginine vasopressin; PRA, plasma renin activity; ANOVA, analysis of variance; df, degrees of freedom. * $P < 0.05$.

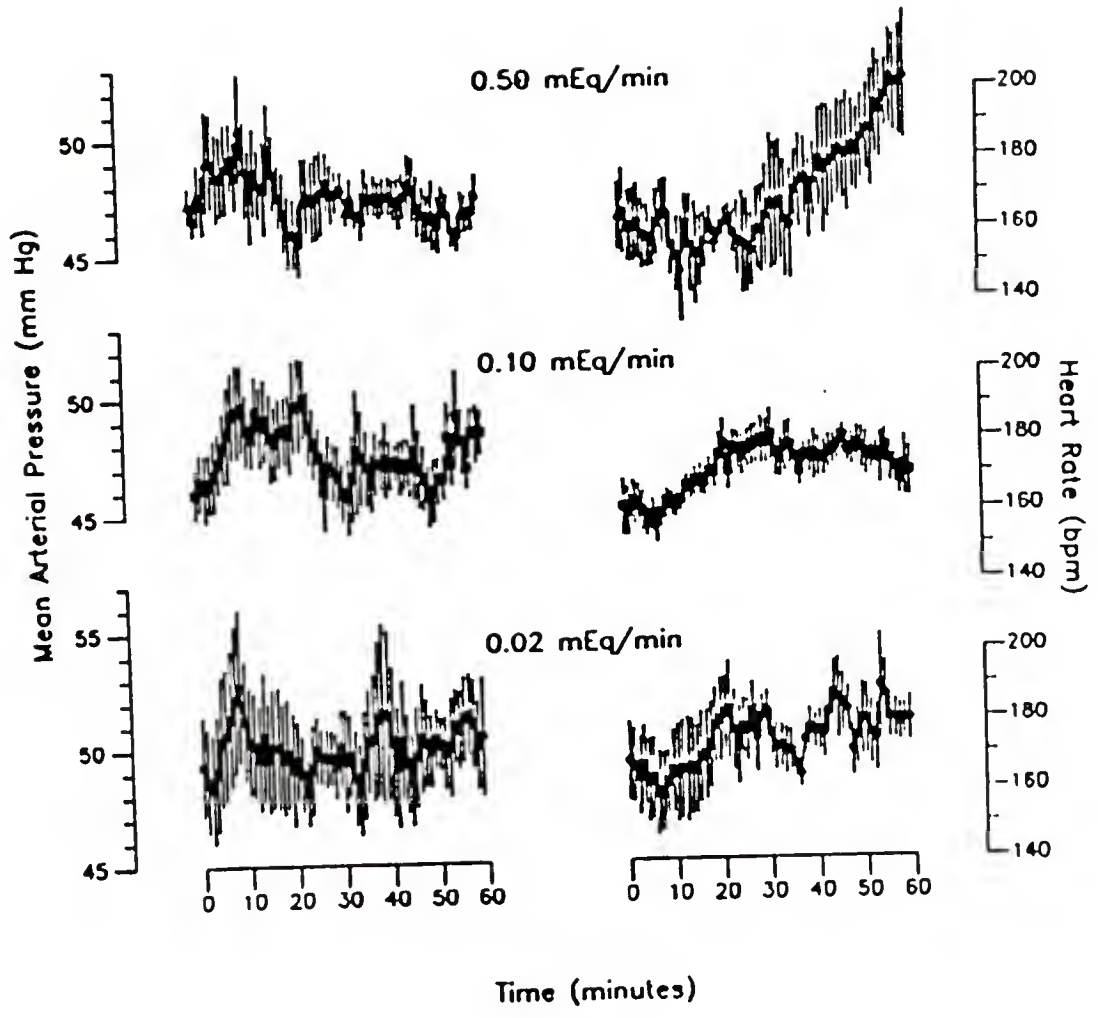

Figure 5-2. Fetal MAP (left) and HR (right) during intravenous infusion of HCl at rates of 0.02 (bottom), 0.10 (middle), and 0.50 (top) meq/min.

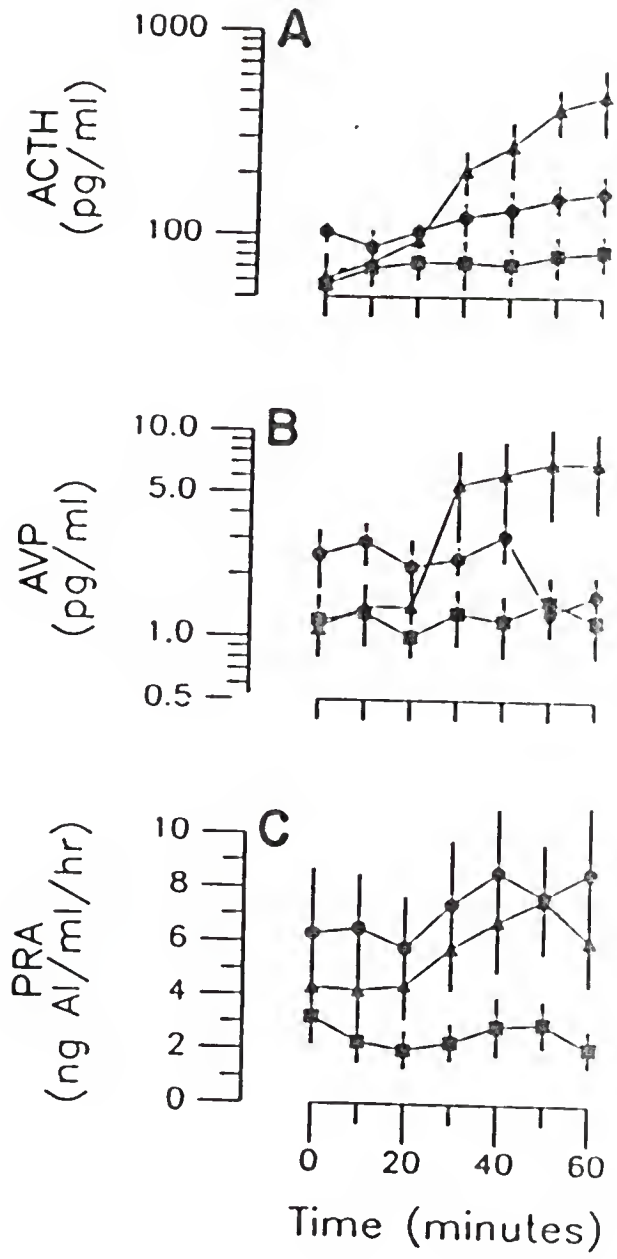

Figure 5–3. Mean fetal plasma ACTH (A), AVP (B), and PRA (C) during intravenous infusion of HCl at rates of 0.02 (filled circles), 0.10 (filled squares), and 0.50 (filled triangles) meq/min.

Table 5–2. Fetal plasma cortisol concentrations

Time, min	Infusion Rate, meq/min		
	0.02	0.10	0.50
0	26±8	23±2	19±5
10	26±10	22±4	20±4
20	23±6	20±4	19±5
30	29±6	22±4	25±6
40	35±11*	24±6†	27±6
50	31±7	22±4†	31±6*‡
60	33±9	19±4	33±7*‡

Values are means ± SE. * Significantly different from initial time point in that group. † Significantly different from same time point in 0.02-meq/min group. ‡ Significantly different from same time point in 0.1-meq/min group.

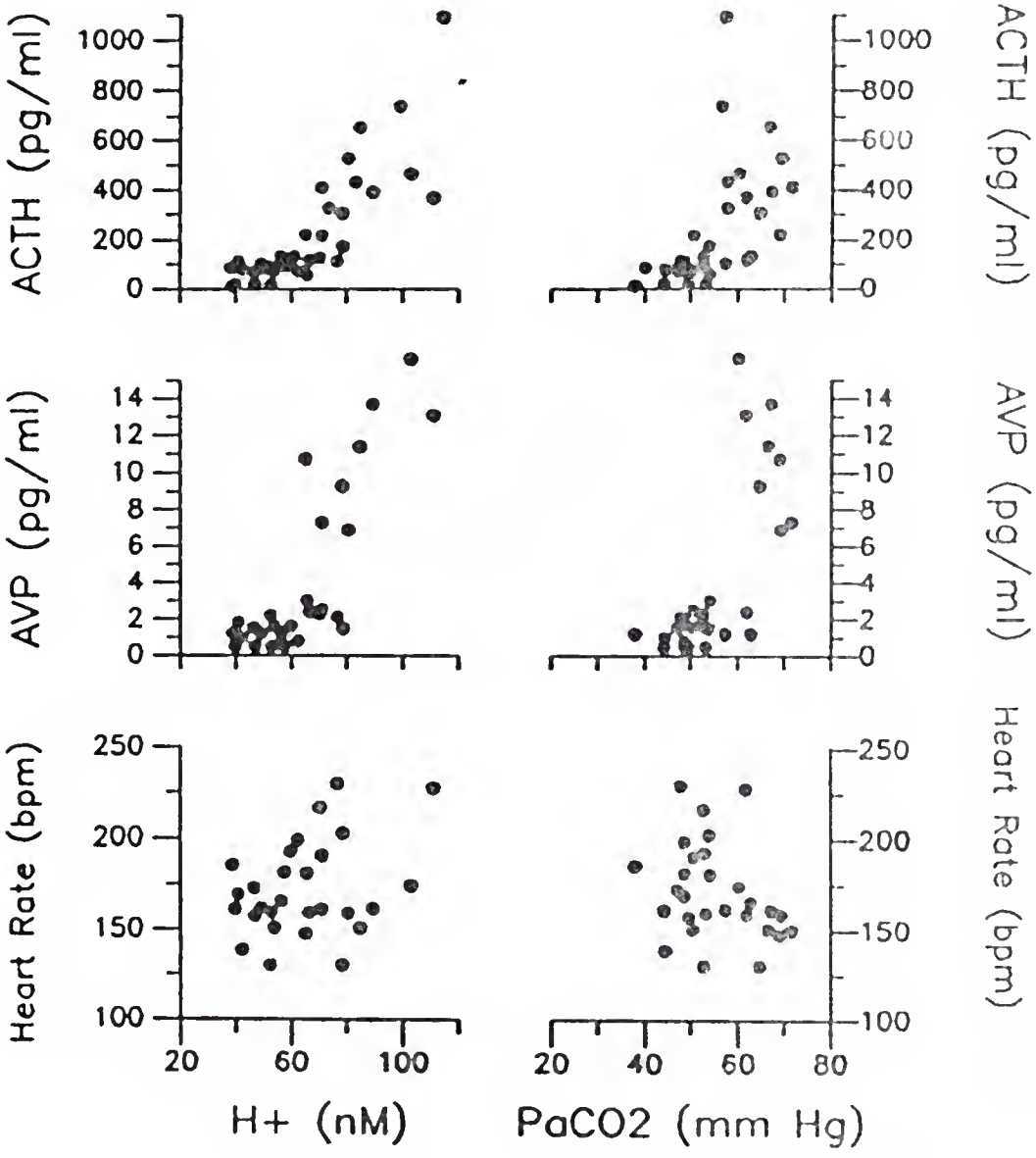

Figure 5-4. Relationships between arterial H^+ concentration (left), Pa_{CO2} (right) and plasma ACTH (top), AVP (middle), and HR (bottom) in 0.50 meq/min group. All points within the 0.50 meq/min group are shown.

fetal plasma ACTH (r=0.742, n=35) and AVP (r=0.754, n=28) were also significantly correlated to the changes in Pa_{CO2}. As shown in Figure 5-4, the relationships between heart rate and pH_a and Pa_{CO2} were much less consistent. This may be related to the inherent variability in fetal heart rate.

Fetal PRA was not stimulated by H^+ (Figure 5-3, Table 5-1). It is likely that PRA increased equally in all groups in response to the blood sampling. The logarithm of fetal PRA was not correlated significantly to the induced changes in pH_a (r=-0.007, n=35) or Pa_{CO2} (r=-0.238, n=35) during infusion of 0.5 meq/min. Initial values of PRA were not different among groups (1-way ANOVA).

Fetal hematocrit was also significantly altered by the infusions, although the effect was small (Table 5-3). A posteriori comparison of individual means using Duncan's multiple range test indicates a small but significant increase in hematocrit in the 0.02 meq/min group at 50 min relative to the initial value in that group (mean change in hematocrit=-2%) and a significant increase in hematocrit at 30 min in the 0.5 meq/min group at 30 min relative to the initial value in that group (mean change in hematocrit=+1%). The hematocrit values were significantly lower in the 0.02 meq/min group at all time points compared with the other two groups.

5.4 Discussion

The results of this study demonstrate that fetal ACTH and AVP secretion are stimulated by fetal metabolic acidemia in fetal sheep between 132 and 140 days gestation. In a previous study, we found that ACTH, AVP, and renin responses to slow hemorrhage were significantly correlated to decreases in pH_a and increases in Pa_{CO2} in fetal sheep between 128 and 132 days gestation (Chapter 4). The results of these two studies cannot be directly compared because of the differences in gestational ages of the fetuses. However the results

Table 5–3. Fetal hematocrit

Time, min	Infusion Rate, meq/min		
	0.02	0.10	0.50
0	38±1*	41±2	41±1
10	38±1*	41±2	40±1
20	38±1*	41±2	41±0
30	38±1*	40±2	42±1†
40	37±1*	40±2	40±1
50	36±2*†	40±2	40±0
60	37±1*	40±2	40±0

Values are means ± SE. * Significantly different from same time point in other groups. † Significantly different from initial value in that group.

of the present study suggest that fetal ACTH and AVP responses to fetal hemorrhage might be at least partially mediated by increases in fetal Pa_{CO2} or decreases in pH_a.

In adult animals, acute metabolic acidemia (produced by intravenous or intraduodenal infusion of H^+) increases plasma concentrations of both glucocorticoid and mineralocorticoids (Augustinsson and Johansson, 1986; Perez et al., 1979). Chronic metabolic acidosis, produced by diabetic ketoacidosis, is also associated with increased plasma concentrations of cortisol, aldosterone, AVP, and renin (Christlieb, 1976; Christlieb et al., 1975; Gerich et al., 1971; Walsh et al., 1979). However, the hormonal responses to diabetic ketoacidosis may be in part related to the associated hypovolemia (Waldhausl et al., 1979).

In fetal animals, acute acidemia is known to increase plasma concentrations of AVP and epinephrine (Faucher et al., 1987). In that study, the investigators infused NH_4Cl into fetal sheep at 137±4 (SD) days gestation at a rate of 0.382 meq/min for 120 min. That rate of acid infusion decreased fetal pH_a from 7.37±0.01 to 7.04±0.05 at the end of the 120-min infusion but did not alter fetal Pa_{O2} and Pa_{CO2}. The acidemia stimulated an increase in fetal plasma AVP from 2.85 ± 0.23 to 5.26 ± 1.11μU/ml during the 120-min infusion period. In the present study, the infusion of HCl at a rate of 0.5 meq/min for 60 min. The rate of HCl infusion increased Pa_{CO2}, as well as decreasing pH_a, and increased plasma AVP. We found that this infusion of HCl increased plasma AVP from 1.1±0.3 to 7.0±3.0 pg/ml, a nearly seven fold increase. While it is not valid to compare absolute values for plasma AVP between laboratories, it is apparent that Faucher and co-workers (Faucher et al., 1987) observed only an approximately twofold increase in plasma AVP during their acid infusion study. The major difference between our study and theirs is that our infusion of HCl increased Pa_{CO2}, while their infusion of NH_4Cl did not. This was probably related to the higher rate of infusion and the stronger acid used in our experiments. Together, these studies may indicate that there is a significant interaction between acidemia and hypercapnia in the control of AVP secretion in the fetus. Whether this apparent effect of hypercapnia is mediated by the

peripheral or central chemoreceptors is not known. Another difference between these studies is the rate of change of pH_a. In the present study, pH_a decreased at an apparently linear rate of 0.36 pH units/h, and in the Faucher study pH_a decreased at an apparently linear rate of 0.17 pH_a units/h. The possible influence of the rate of change of pH_a on AVP secretion is unknown at the present time.

Fetal heart rate was increased by infusion of HCl at a rate of 0.5 meq/min. Stimulation of peripheral fetal chemoreceptors by fetal intravenous cyanide injection or occlusion of the maternal descending aorta produces reflex bradycardia (Iskovitz and Rudolph, 1982). Analogously, chemoreceptor stimulation in adult animals produces bradycardia if ventilation is maintained constant(i.e., if the reflex tachypnea is blocked). If reflex tachypnea is allowed in adult animals after stimulation of the chemoreceptors, one observes tachycardia, indicating an interaction between the chemoreceptor and pulmonary afferent fibers in the control of heart rate. It is possible that in the present experiments the fetal sheep developed tachycardia during HCl infusion of 0.5 meq/min because they also started fetal breathing movements. We did not measure fetal breathing movement in this study; however, it is known from other studies that hypercapnia stimulates fetal breathing movement (Walker, 1984b). In summary, it is therefore possible that infusion of HCl at a rate of 0.5 meq/min stimulated increases in fetal heart rate because of increased stimulation of chemoreceptors combined with stimulation of fetal breathing movement. Fetal heart rate during acid infusion was also measured in a study by Widness and co-workers (1986). In that study, infusion of lactic acid at a rate of 0.58–0.75 meq/min decreased fetal arterial pH from 7.37 to 7.21 over the course of 4.5 hour. The rate of change of pH_a was lower (0.04 pH units/h) than in the present study. There were no significant changes in fetal heart rate or P_{aCO2}. It is possible that the changes in heart rate and Pa_{CO2} in the present study were partially dependent on the rate of change of pH_a.

Infusion of acid into the fetal circulation increased fetal Pa_{CO2}. In adult animals with spontaneous ventilation, arterial acidemia reflexly increases ventilation, decreasing Pa_{CO2}. The fetal animal controls gas exchange by altering umbilical–placental blood flow. In the present experiments, it is apparent that the Pa_{CO2} increased because umbilical–placental blood flow did not increase accordingly.

We conclude that acute acidemia is a potent stimulus to ACTH and AVP secretion in fetal sheep between 132 and 140 days gestation. The lack of response of PRA to the acidemia may indicate insensitivity of renin to control by changes in pH_a or Pa_{CO2} or may have been the result of secondary inhibition of renin secretion by the increased Cl^- load at the fetal macula densa (Share and Levy, 1962). These results support the hypothesis that the acidemia produced during hemorrhage might partially stimulate the ACTH and AVP responses to the hemorrhage.

CHAPTER 6
REFLEX CONTROL OF FETAL ARTERIAL PRESSURE AND HORMONAL RESPONSES TO SLOW HEMORRHAGE

6.1 Introduction[3]

Fetal sheep respond to slow hemorrhage with increases in fetal plasma ACTH and AVP concentrations and PRA (Alexander et al., 1974; Brace and Cheung, 1986; Drummond et al., 1980; Ross et al., 1986). From Experiment I (Chapter 4), we found that bilateral vagotomy, which interrupts afferent fibers from atrial type B receptors did not alter the ACTH, AVP or PRA responses to hemorrhage. The receptors mediating the hormonal responses to mildly hypotensive hemorrhage are therefore different in the fetus than in the adult (Cryer and Gann, 1974; Share and Levy, 1962). We and others have demonstrated that the ACTH, AVP, and renin responses to hemorrhage were significantly correlated to changes in pH_a, but were not correlated to changes in arterial or venous pressure. From Experiment II, we found that hypercapnic acidemia, produced by infusion of hydrochloric acid increased fetal ACTH and AVP (Chapter 5). Together, the results of these studies suggest that ACTH and AVP responses to slow hemorrhage might be chemoreceptor-mediated.

The present study was designed to investigate the role of peripheral chemoreceptors in controlling the fetal hormone secretion during slow hemorrhage. To test this hypothesis, we compared responses to slow hemorrhage in intact fetuses to responses in fetuses subjected to bilateral vagotomy combined with bilateral carotid sinus denervation. The combined

[3] This work was supported by the National Institute of Health grant HD-20098.

denervation impaired the ability of the fetus to defend blood pressure during the period of hypovolemia, exaggerating the changes in fetal arterial blood gases usually produced by hemorrhage. We therefore added a third group, denervation plus infusion of phenylephrine (an ∝-adrenergic agonist drug) to support blood pressure and umbilical-placental perfusion during the period of hypovolemia. Comparison of these three experimental groups allows assessment of the roles of carotid sinus and vagal afferents together in the control of reflex hormonal and hemodynamic responses to slow hemorrhage in the late-gestation fetal sheep.

6.2 Methods

6.2.1 Experimental Protocol

Twenty-six chronically catheterized fetal sheep of 121-138 days of gestation were studied. Four of them were twins. Nineteen of these fetuses were subjected to bilateral section of cervical vagosympathetic trunks and the carotid sinus nerves in order to deafferent peripheral chemoreceptors and baroreceptors.

All fetuses underwent the same 120-minute hemorrhage procedure, and each fetus was studied once. At ten-minute intervals, 11 ml blood was withdrawn from a fetal arterial catheter. One ml of the blood was drawn anaerobically for analysis of fetal pH_a, Pa_{O2}, Pa_{CO2}, plasma protein, plasma sodium, potassium and Hct and 10 ml were used for hormone analysis. Therefore, the total volume of blood withdrawn was 143 ml. One maternal arterial blood sample was withdrawn at the beginning of each experiment. Each fetus was studied once.

The fetuses were divided into three groups: 1) intact fetuses (n=7); 2) denervated fetuses (n=12); 3) denervated fetuses infused intravenously with phenylephrine hydrochloride (Winthrop-Breon Laboratories, Division of Sterling Drug, Inc.) using a syringe pump (Sage Instruments model 341, Cambridge, MA). The rate of phenylephrine infusion was adjusted to maintain the fetal blood gases at pre-hemorrhage levels. Infusion was started during the

period between 30 to 40 minutes after the onset of the hemorrhage; the rate of infusion was varied between 0.01 ml/min and 0.38 ml/min (0.2–7.6 μg/ml). All ewes and fetuses were sacrificed and the fetuses were weighed immediately after the experiments.

6.2.2 Calculated Variables

All of the following calculations were performed as described by Brace and coworkers (Brace and Cheung, 1986; Brace, 1983).

Initial blood volume ($BV_{0)}$ was assumed to be approximately 110 ml/kg, and subsequent blood volume at each time point (BV_t) was calculated from BV_0, Hct of each sample (Hct_t), and volume of blood removed from the fetus as measured by Brace (Brace and Cheung, 1986). Therefore,

$$BV_0 = 110 \text{ ml/kg} \times Wt \qquad [1]$$

and

$$BV_t = BV_0 \times FRCV \times Hct_0/Hct_t \qquad [2]$$

where Hct_o and Hct_t are initial and subsequent hematocrits, respectively, and FRCV is the fractional red cell mass. In this study, FRCV was calculated as follows:

First, calculate the initial red blood cell mass (RBC_0):

$$RBC_0 = BV_0 \times Hct_0/100$$

Then, subsequent RBC remaining (RBC_t) at each 10–min interval of hemorrhage was calculated as:

RBC_t = Initial RBC – RBC removed during hemorrhage

$= RBC_0 - 11 \times (Hct_0 + Hct_1 + ... + Hct_t)/100$

Thus,

$$FRCV = RBC_t / RBC_0$$

V_r and R_f are referred to as the blood volume removed and the blood volume restitution during hemorrhage. We have

$$V_r = 11 \times n$$

and

$$R_f = V_r - (BV_0 - BV_t)$$

where n is the number of 11 ml samples. Therefore, the %R after hemorrhage was calculated using the following equations (Brace, 1983):

$$\%R = R_f/V_r \times 100 \quad [3]$$

Assuming that the fluid entering the circulation contained no protein, the percent change of the calculated or theoretical plasma protein concentration ($\%\Delta P_c$) was calculated (Brace, 1983) by

$$\%\Delta P_c = 100 \times (Hct_0 - Hct_t)/(100 - Hct_0) \quad [4]$$

The $\%\Delta P_a$ was calculated by

$$\%\Delta P_a = (P_0 - P_t)/P_0 \times 100 \quad [5]$$

where P_0 is the plasma protein concentration before hemorrhage and P_t is the protein concentration at each time point.

The protein concentration in restitution fluid (P_{rf}) was calculated by

$$P_{rf} = (P_t - P_c) \times BV_t/R_f \quad [6]$$

where

$$P_c = (1 + \%\Delta P_c) \times P_0$$

6.3 Results

Initial values of all fetal blood gases, blood pressures, HR and fetal and maternal hormone concentrations were similar to values previously reported for healthy fetuses and ewes in this laboratory (See previous chapters), suggesting that the fetuses had recovered from surgery. Initial values of variables were not significantly different among experimental groups as tested by one-way ANOVA.

Maternal plasma ACTH concentrations in intact, denervated, and denervated plus phenylephrine infusion groups were 103±40, 167±39, and 120±41 pg/ml, respectively, and maternal plasma cortisol concentrations in these groups were 11.7±2.5, 13.3±2.1, and 9.6±1.5 ng/ml, respectively. Maternal plasma vasopressin concentrations in the three groups were 0.8±0.2, 4.3±1.2, and 3.4±1.2 pg/ml, and plasma renin activities were 2.7±1.1, 1.7±0.2, and 2.0±0.8 ng Ang I/ml•hr, respectively. None of the measured maternal plasma hormone levels differed significantly among groups, as tested by one-way ANOVA.

6.3.1 Changes in Hemodynamic Variables During Hemorrhage

Hemorrhage decreased fetal MAP in both intact (Group 1) and denervated (Group 2) fetuses. The decrease in MAP was more severe in the denervated fetuses (Figure 6-1, top

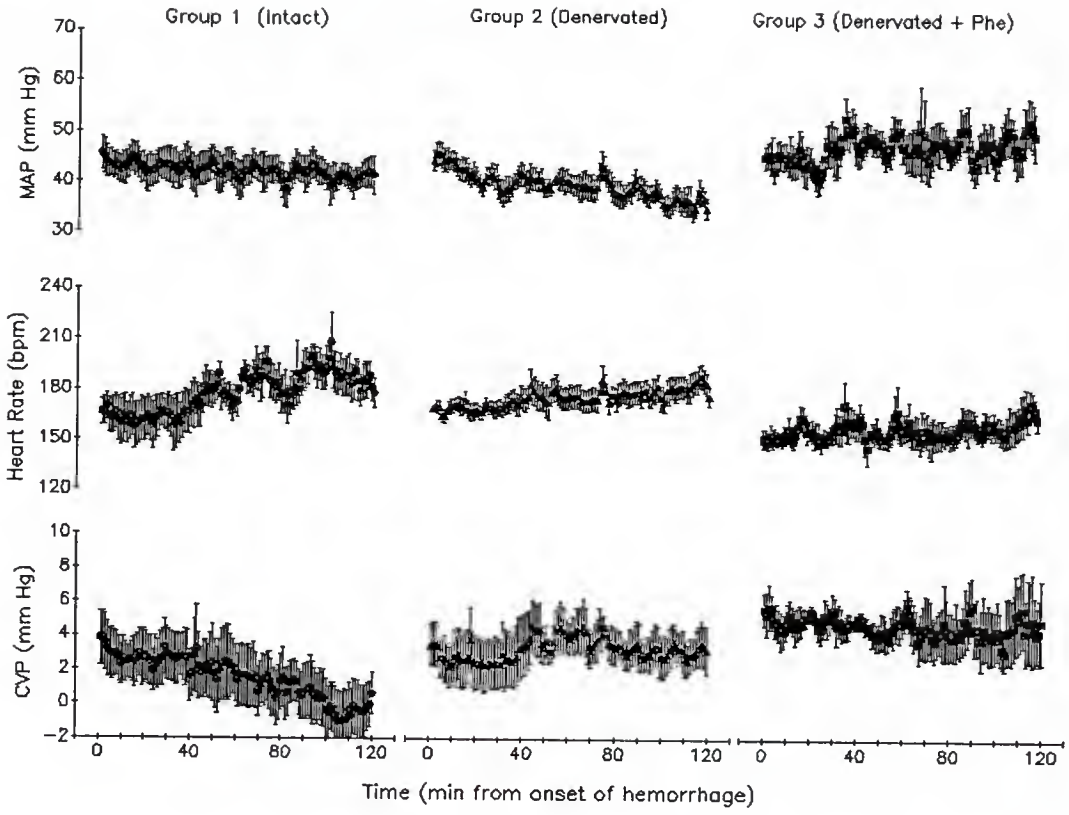

Figure 6–1. MAP (top), HR (middle) and CVP (bottom) during hemorrhage in fetal sheep which were intact (left), subjected to bilateral sino-aortic and vagosympathetic denervation (middle), and subjected to denervation plus phenylephrine infusion (right).

panel). Infusion of phenylephrine increased arterial blood pressure during hemorrhage in denervated fetuses (Group 3).

Heart rate increased in all groups during hemorrhage (Figure 6–1, middle panel). There were no significant differences among groups. Fetal CVP was significantly decreased during hemorrhage in intact fetuses (Figure 6–1, bottom panel).

6.3.2 Changes in Blood Gases During Hemorrhage

The initial values of Pa_{CO2} and pH_a were not significantly different. Hemorrhage significantly decreased fetal pH_a and increased Pa_{CO2}. The changes of pH_a and Pa_{CO2} produced by the hemorrhage were greatly exaggerated by denervation; infusion of phenylephrine restored the pH_a response to a magnitude that was not different from that in intact fetuses. Pa_{O2} was not significantly altered by the hemorrhage (Figure 6–2, top panel).

6.3.3 Changes in Plasma Hormone Levels

Hemorrhage stimulated fetal ACTH and cortisol secretion in all three groups compared to either intact or denervated fetuses infused with phenylephrine (Figure 6–3, left panel). The responses were greatest in denervated (Group 2) fetuses. Infusion of phenylephrine attenuated these responses such that the magnitudes of the responses were similar to those of the intact fetuses. Plasma ACTH concentration was significantly higher than the other two groups after 70 min in denervated fetuses. Plasma ACTH concentration in Group 3 (denervated plus infusion of phenylephrine) was lower than the concentration in Group 1 (intact) at 120 min. Plasma cortisol concentration was higher in denervated fetuses after 20 min than in either of the other two groups. There were no significant increases in cortisol above initial concentrations in either group 1 or group 3.

Hemorrhage stimulated fetal AVP secretion in both intact and denervated fetuses. Phenylephrine infusion abolished the AVP response to hemorrhage in denervated fetuses

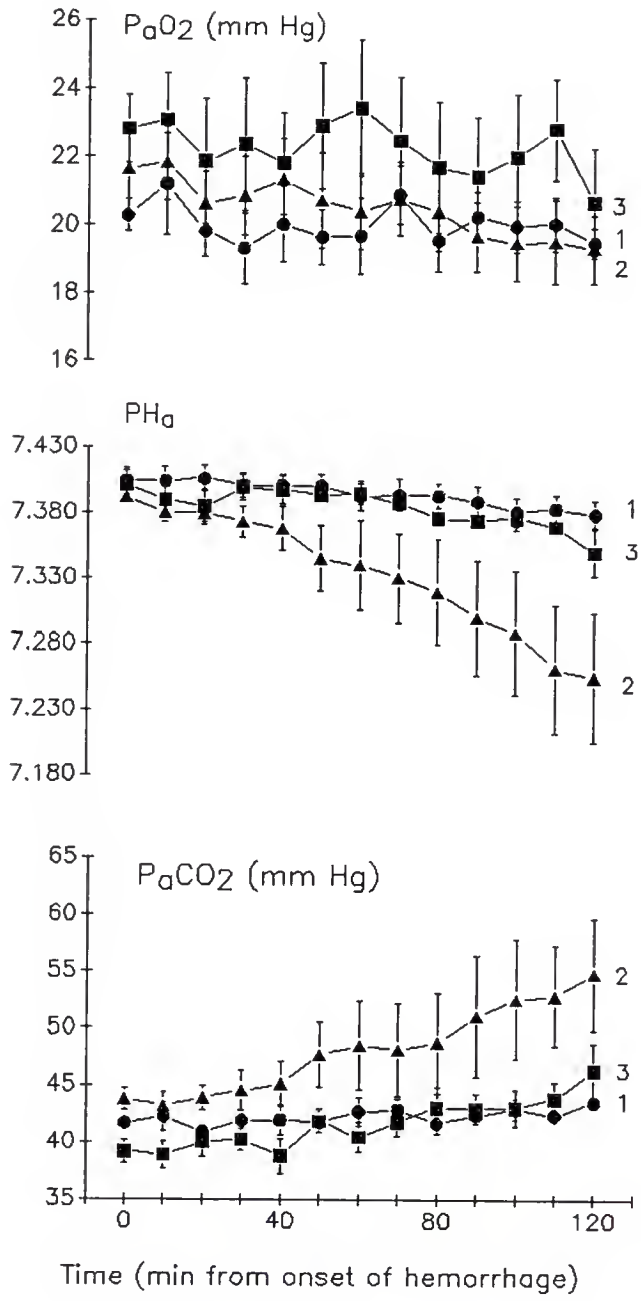

Figure 6–2. Pa_{O2}, pH_a and Pa_{CO2} during hemorrhage in fetal sheep which were intact (filled circles), denervated (filled triangles) and denervated plus phenylephrine infusion (filled squares).

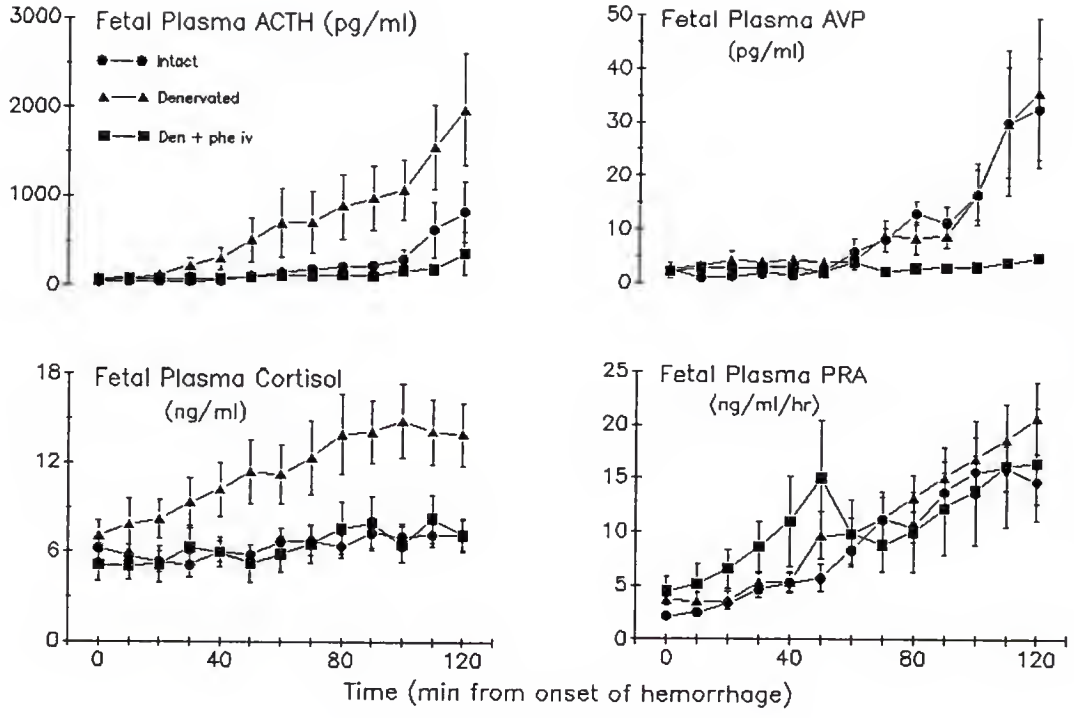

Figure 6-3. Fetal plasma ACTH (top left), cortisol (bottom left), AVP (top right) and PRA (bottom right) during hemorrhage in fetal sheep which were intact (filled circles), denervated (filled triangles) and denervated plus phenylephrine infusion.

(Figure 6–3, right top panel). There was a significant suppression of AVP in the denervated fetuses infused with phenylephrine after 110 min relative to the denervated fetuses not infused with phenylephrine.

Hemorrhage stimulated similar PRA responses in all 3 groups (Figure 6–3, right bottom panel).

6.3.4 Blood Volume Restitution

All fetuses were bled the same absolute volume of blood. The ratio of hemorrhage volume to estimated initial blood volume was not different among groups: 47.0 ± 0.1%, 47.0 ± 0.1% and 53.7 ± 0.1% for Groups 1, 2, and 3, respectively. Figure 6–4 shows that intact fetuses restored blood volume better than the fetuses in the two denervation groups. The effect of the denervation on blood volume restitution is summarized in Table 6–1. Denervation reduced the final restitution of blood volume 21.2% (calculated from volumes expressed as ml/kg) or 23.4% (calculated from volumes expressed as the percentage of hemorrhage volume). Infusion of phenylephrine did not restore the rate of blood volume restitution observed in the intact fetuses.

Plasma protein concentrations decreased in all three groups, (Figure 6–5, left bottom). The decrease in plasma protein concentration suggests that transcapillary refilling occurred with fluid containing protein at a concentration less than that of plasma. Figure 6–6 illustrates the percentage change in plasma protein that was measured and the calculated change assuming there is no protein in the fluid entering the vascular space. The values were significantly different between measured and calculated values within each group. This analysis can be used to calculate the mean protein concentration in the fluid entering the vascular space. These values are reported in Table 6.1. The protein concentrations range from 2.7 ± 0.4 to 3.7 ± 0.2 g/dl, and are not significantly different among groups.

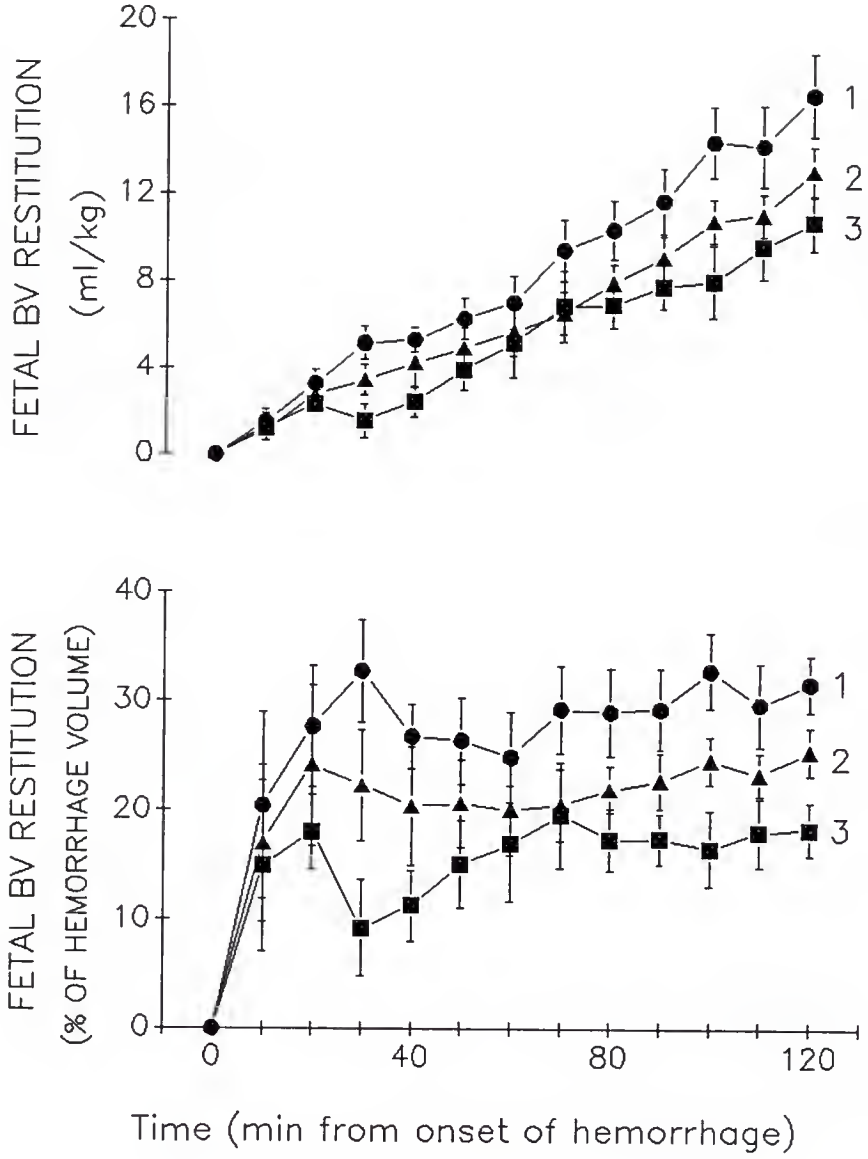

Figure 6-4. Fetal volume restitution, which is represented as ml/kg (top) and percentage changes of hemorrhage volume (bottom). Filled circles, triangles and squares are represented intact, denervated and denervated plus phenylephrine infusion groups.

Table 6-1. Initial and final fetal blood volume and fetal blood volume restitution.

	INTACT	DEN@	DEN+PHE@
INITIAL BV (ml) (estimated)	309.0 ± 15.0	319.0 ± 22.0	273.0 ± 17.0
FINAL BV (ml) (estimated)	211.0 ± 14.0	213.0 ± 22.0	156.0 ± 18.0
FINAL HEMORRHAGE RATIO (% of BV_0)	47.0 ± 0.1	47.0 ± 0.1	53.7 ± 0.1
FINAL BV RESTITUTION (ml/kg)	16.5 ± 1.9	#13.0 ± 1.2	#*10.7 ± 1.3
FINAL BV RESTITUTION (% of hem. volume)	31.5 ± 2.6	25.4 ± 2.2	#*18.3 ± 2.4
FINAL PROTEIN CONC. IN RESTITUTION FLUID (g/dl)	3.7 ± 0.2	3.5 ± 0.6	2.7 ± 0.4

Duncan's multiple test shows significant difference from intact fetuses.

* One-way ANOVA shows significant difference among groups.

@ DEN: denervated fetuses; DEN + Phe: denervated plus phenylephrine infusion

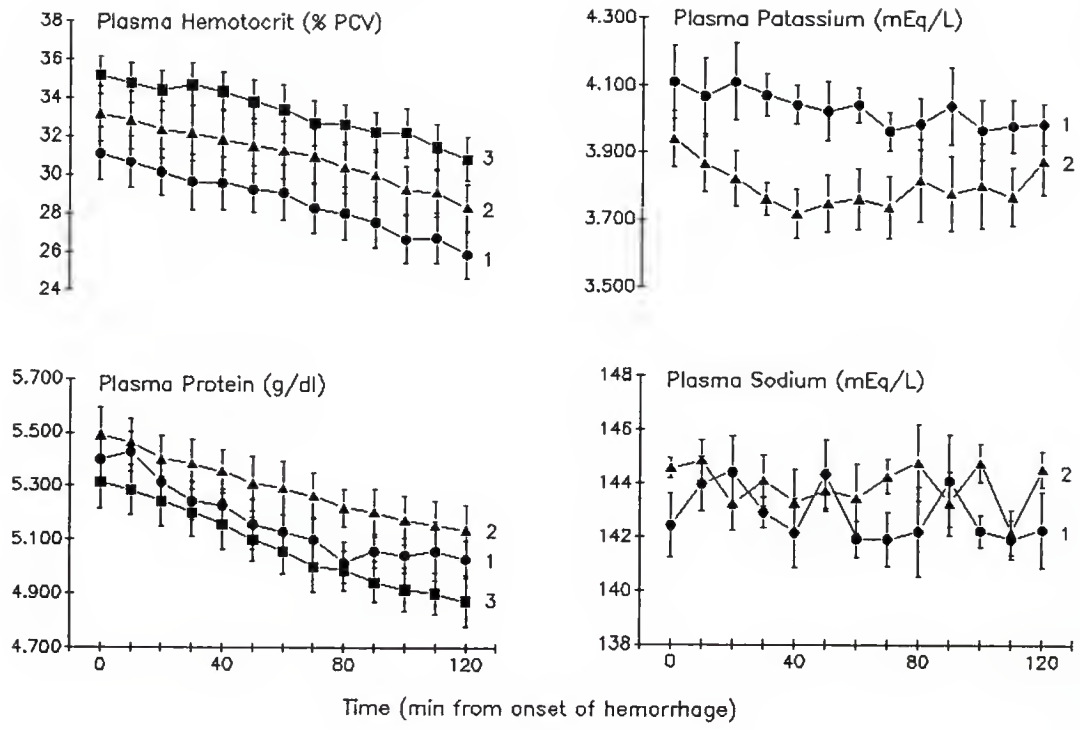

Figure 6–5. Fetal Hct (top left), plasma protein (bottom left), potassium (top right) and sodium (bottom right) in fetuses which were intact, denervated and denervated plus phenylephrine infusion (filled circles, triangles and squares, respectively).

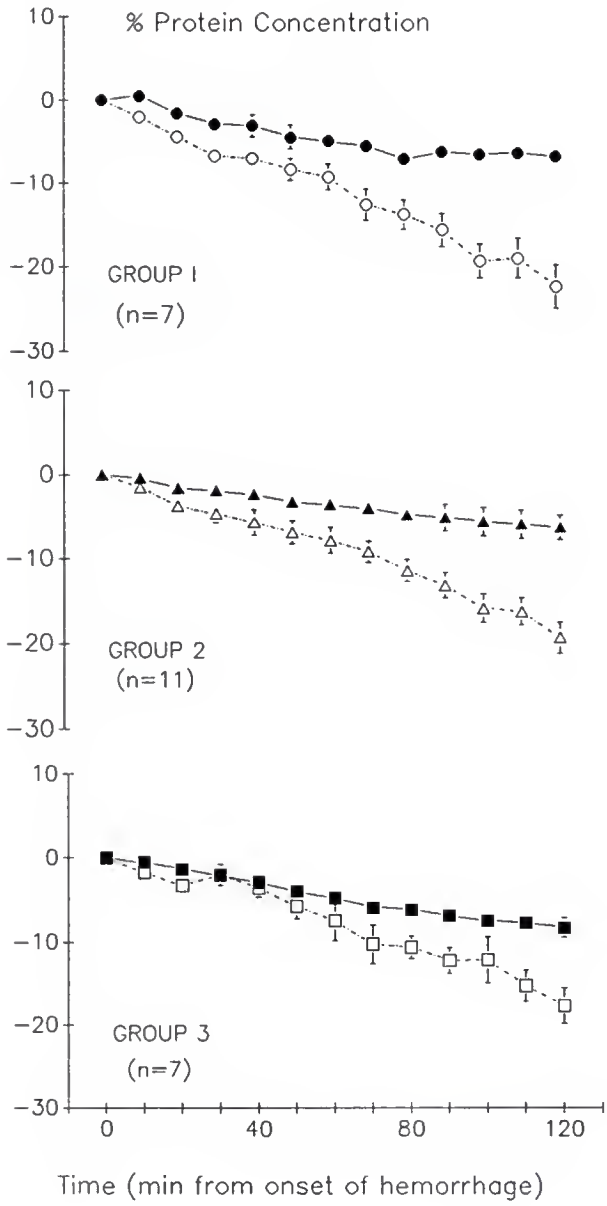

Figure 6-6. Percent changes in fetal plasma protein concentration. Open symbols represent the theoretical changes and the filled symbols represent the actual changes.

Plasma potassium and sodium concentrations were only measured in 7 intact (Group 1) and 11 denervated (Group 2) fetuses (Figure 6–5, right). There was a tendency for plasma potassium concentration to decrease during hemorrhage, but the apparent changes were not statistically significant. There were also no significant differences in plasma sodium concentrations between groups.

6.4 Discussion

This study was designed to test the role of peripheral chemoreceptors and cardiovascular mechanoreceptors in the control of the hormonal and cardiovascular responses to slow hemorrhage in the late gestation fetal sheep. We found that section of the vagosympathetic trunks plus bilateral denervation of the carotid arterial baroreceptors and chemoreceptors had a profound effect on the responses to hemorrhage. We successfully controlled the exaggerated changes in blood gases during hemorrhage by infusing phenylephrine (an α–adrenoreceptor agonist) into the denervated fetuses during hemorrhage to constrict the fetal systemic vasculature, and to increase placental flow to maintain changes in fetal arterial blood gases that were similar to those observed in the intact fetuses. The infusion of phenylephrine did not, however, improve BV restitution in denervated fetuses. The phenylephrine infusion attenuated the ACTH and cortisol responses and blocked the AVP response, but did not change the PRA response.

6.4.1 Alterations in Blood Gases and Vascular Pressures

The greater decreases in MAP and pH_a and the greater increase in Pa_{CO2} suggested that the afferent fibers sectioned in the denervated compared to intact fetuses are important in the reflex vasomotor responses to hemorrhage. In a previous study, we compared responses to hemorrhage in intact and vagotomized only fetuses and the later were not significantly

different from the responses in intact fetuses. Therefore, it is likely that afferent fibers from the carotid sinus are critical for maintaining arterial pressure and blood gases during hemorrhage. It is also possible that interruption of fibers important for maintenance of arterial blood pressure during hypovolemia requires both vagotomy and carotid sinus denervation, since we did not test the response to hemorrhage in fetuses subjected to carotid sinus denervation alone.

The large changes in fetal arterial blood gases in the denervated fetuses not infused with phenylephrine was most probably the result of reduced flow in the umbilical–placental vascular bed secondary to reduced arterial blood pressure. Umbilical–placental flow is influenced by the relative distribution of fetal combined ventricular output between the umbilical–placental vascular bed and other vascular beds in the fetus (Itskovitz et al., 1982; Faber et al., 1973; Perez et al., 1989). Reflex control of blood pressure and blood gases during hemorrhage involves vasoconstriction in the fetal body and redistribution of combined ventricular output to the placenta. The results of these and other experiments demonstrate that the umbilical–placental vascular bed is less sensitive to alpha–adrenergic stimulation than other vascular beds in the fetus (Berman, 1978; Oakes et al., 1980). Thus, infusion of phenylephrine during hemorrhage in the denervated fetuses redistributes flow to improve gas exchange.

Central venous pressure did not decrease as much in the totally denervated fetal sheep as much as in the intact fetal sheep. It is possible that, if the denervation partially blocked the reflex sympathetic efferent response to the hemorrhage, cardiac contractility would not have been as high in the denervated fetuses as in the intact fetuses. We therefore speculate that central venous pressure does not fall during hemorrhage in the totally denervated fetuses because of a reduced of cardiac output compared to the intact fetuses.

6.4.2 Reflex Hormonal Responses

ACTH, AVP, and PRA were increased in the intact fetuses. The magnitudes of the responses appeared related to the changes in pH_a and Pa_{CO2}, confirming results of previous studies by us and others (Challis et al, 1989; Faucher et al, 1987; Chapter 4 and 5). However, none of the responses were inhibited or attenuated by denervation, which suggests that the sectioned fibers were not the sole mediators of the hormonal responses. In other experiments, severe hypercapnic acidemia caused by HCl infusion in intact fetuses (pH_a from 7.397±0.008 to 7.038±0.038; Pa_{CO2} from 43.1±1.8 to 57.5±3.2 mm Hg) increased both ACTH and AVP secretion (Chapter 5). Therefore, assuming that the responses to hypotension in the intact fetus are not mediated by cerebral ischemia, central chemoreceptors might mediate part of the AVP response to slow hemorrhage. Together, these results prove that severe hypercapnic acidemia is a stimulus to ACTH and AVP secretion. It is likely that peripheral chemoreceptors do not mediate either the AVP or the ACTH response to slow hemorrhage.

The role of central chemoreceptors in the regulation of fetal ACTH and AVP secretion might be greater during hemorrhage than during metabolic acidemia. Central chemoreceptors might have been greatly stimulated by the combination of the exaggerated increase in P_aCO_2 and a possible reduction in cerebral blood flow in the denervated group caused by hypotension. The augmented ACTH and the unchanged AVP responses in the denervated group were either attenuated or inhibited after phenylephrine infusion. It is possible that phenylephrine removed the remaining stimulus to central chemoreceptors by normalizing the blood gas responses and increasing cerebral blood flow during the hemorrhage. Alternatively, it is also possible that cardiac mechanoreceptors with non-vagal afferent fibers contributed to the ACTH and AVP response to hemorrhage after denervation might reflect the different sensitivity of each receptor not deafferented by the vagotomy and carotid sinus denervation. Finally, it is not likely that osmoreceptors stimulated AVP secretion in current study.

Although we did not measure plasma osmolality, plasma sodium concentration did not change, suggesting little change in plasma osmolality.

PRA responses to the hemorrhage were not altered by denervation or by infusion of phenylephrine in the denervated fetuses. These results do not, by themselves, rule out the control of renin secretion by peripheral or central chemoreceptors. However, changes in pH_a and Pa_{CO2} are not potent stimuli to renin secretion (Wood et al., 1989). We have demonstrated that, although PRA responses to hypercapnia, isocapnic hypoxia, or hypercapnic hypoxia are statistically significant, the responses are smaller in magnitude than the responses to hemorrhage. PRA responses to hypercapnia are attenuated by sinoaortic denervation (Wood et al., 1989). Together, the results of this and our previous studies suggest that the renin response to hemorrhage is only partially mediated by peripheral chemoreceptors. Alternatively, it is possible that PRA responses are also influenced by other factors, such as humoral substances like atrial natriuretic peptide (Scheuer, 1987), and other cardiovascular mechanoreceptors that are not identical with the carotid sinus or aortic arch mechanoreceptors, and do not have afferent fibers in the vagosympathetic trunks.

6.4.3 Defense of Blood Volume

The reduction of fetal hematocrit during hemorrhage reflects the restitution of blood volume. Since under normal conditions the placenta is quite impermeable to molecules even as small as sodium (Schroder, 1982), the restitution fluid is thought to come mostly from the fetal interstitial space (Robillard et al., 1979; Brace, 1983). The calculated protein concentration in restitution fluid and the lack of change in the plasma sodium and potassium concentrations in the present study support this notion. However, it is also possible that during hypovolemia the permeability of the placenta increases, allowing transplacental inflow of some fluid from the mother to the fetus (Manku et al., 1975). The high protein

concentration in restitution fluid is also consistent with increased lymphatic return or decreased protein leakage from the circulation.

The average volume restitution in the intact group, expressed as the percentage of the hemorrhage volume, is about 31.5% (ranged from 28.9% to 34.1%). These values are less than those calculated in Brace's studies, approximately 50% (Brace and Cheung, 1986; Brace, 1989). This might be related to the shorter observation period in the present experiments, and related to the probable underestimation of fetal body weight before hemorrhage.

In conclusion, the results of the present experiment demonstrate that carotid sinus baroreceptors or chemoreceptors are important for the maintenance of blood pressure and blood gases during hemorrhage. The results of this and other studies from this laboratory indicate that PRA responses to slow hemorrhage might be partially mediated by peripheral chemoreceptors, and that AVP and ACTH responses are not mediated by peripheral chemoreceptors. Elucidation of the possible role of the central chemoreceptors or other baroreceptor populations needs further investigation.

CHAPTER 7
THE ACTH AND AVP RESPONSES TO NORMOXIC HYPERCAPNIA IN FETAL AND MATERNAL SHEEP

7.1 Introduction[4]

The fetal hypothalamus–pituitary–adrenal axis and neurohypophysis are functional beginning early in fetal life. The activity of these endocrine axes is increased by various hemodynamic stimuli, starting at approximately 90 days gestation (Alexander et al., 1972) and are important for intrauteral survival during fetal stress.

From Experiment I and III (Chapters 4 and 6, respectively), we have demonstrated that slow hemorrhage stimulates plasma ACTH, cortisol and vasopressin secretion in late gestation fetal sheep. In Experiment I, bilateral vagotomy showed no effect on hormonal responses; the hormonal responses were significantly correlated to changes in arterial pH_a, but not to changes in arterial or venous pressure, which suggested that the ACTH and AVP responses during hemorrhage might be chemoreflex–mediated. In Experiment II, severe hypercapnic acidemia, produced by intravenous infusion of hydrochloride acid, stimulated ACTH, cortisol and AVP secretion, suggesting that chemoreceptors might play an important role in mediating fetal hormonal responses to hemorrhage (Chapter 5). While this assumption was disproved by Experiment III (Chapter 6): 1) a more complete peripheral chemodenervation did not attenuate the ACTH, cortisol, AVP and PRA responses to hemorrhage; 2) when the fetal blood pH_a and Pa_{CO2} changes during hemorrhage were

[4] This work was supported by the National Heart, Lung, and Blood Institute grant HL–36289 and American Heart Association.

balanced by infusion of an alpha-agonist, the ACTH, cortisol and AVP increases in denervated fetuses were either attenuated or inhibited. Results from Experiment Series III suggests that peripheral chemoreceptors do not mediate ACTH, AVP or PRA responses to hemorrhage, and a central chemoreceptors was suggested.

This conclusion made us question the assumption we made from Experiment II (Chapter 5), which is: chemoreceptors might mediate ACTH and AVP responses to hemorrhage. Based on the facts that HCl infusion caused metabolic acidemia while slow hemorrhage caused respiratory acidemia, and the former caused much severe acidemia than slow hemorrhage did, We speculated that there must be a threshold for initiating chemoreceptor regulated hormonal responses, and this threshold might not be reached during slow hemorrhage.

The present study was designed to investigate whether mild hypercapnic acidemia, similar in magnitude to the changes during hemorrhage but without the associated hypovolemia, stimulates ACTH and AVP secretion in fetal sheep, and whether these responses are mediated by peripheral chemoreceptors.

7.2 Methods

Twelve fetal sheep of 123-137 days of gestation were studied. All fetuses and their mothers were chronically prepared with catheters and six of these fetuses had been subjected to bilateral section of the cervical vagosympathetic trunks and the carotid sinus nerves in order to deafferent peripheral chemoreceptors and baroreceptors as well as to remove other vagal afferent fibers.

The ewes with their fetuses were subjected to two experiments: normocapnia control and hypercapnia.

A five ml blood samples was withdrawn from both fetal and maternal arterial catheters every 20 minutes for 1 hour. Immediately after drawing the first fetal and maternal blood

samples, a polyethylene bag was fitted over the ewe's head and neck, held loosely in place with elasticized cloth. Normocapnia control was achieved by passing room air through the bag. Hypercapnia was achieved by using a gas mixture of 21% O_2 and between 5% and 8% CO_2 (adjusted to increase fetal Pa_{CO2} at least 10 mm Hg). Data from one of the experiments in each group was eliminated because of the abnormal baseline levels of fetal blood gases or hormones. The order of the experiments performed was randomized.

CVP was not measured in one of the intact fetuses in normocapnia experiment because of the failure of the venous catheters.

7.3 Results

Initial values of all fetal and maternal blood gases, hormones and electrolyte concentrations, fetal blood pressures and HR were similar to values previously reported for healthy fetuses and ewes in this laboratory, suggesting that the fetuses and ewes had recovered from surgery.

7.3.1 Responses in Fetuses

During normocapnia, blood gases, Hct and electrolytes did not change significantly (Table 7-1).

During hypercapnia, pH_a decreased from 7.390 ± 0.001 and 7.398 ± 0.015 to 7.257 ± 0.011 and 7.281 ± 0.010, Pa_{CO2} increased from 36.8 ± 1.4 and 39.8 ± 1.4 to 55.2 ± 1.8 and 55.9 ± 2.2 mm Hg in intact and denervated fetuses, respectively. Pa_{O2} was increased from 25.5 ± 1.4 and 26 ± 1.5 to 31.6 ± 1.9 and 33.6 ± 1.8 mm Hg in intact and denervated fetuses respectively. Changes in blood gases were not significantly different in intact and denervated fetuses (Figure 7-1).

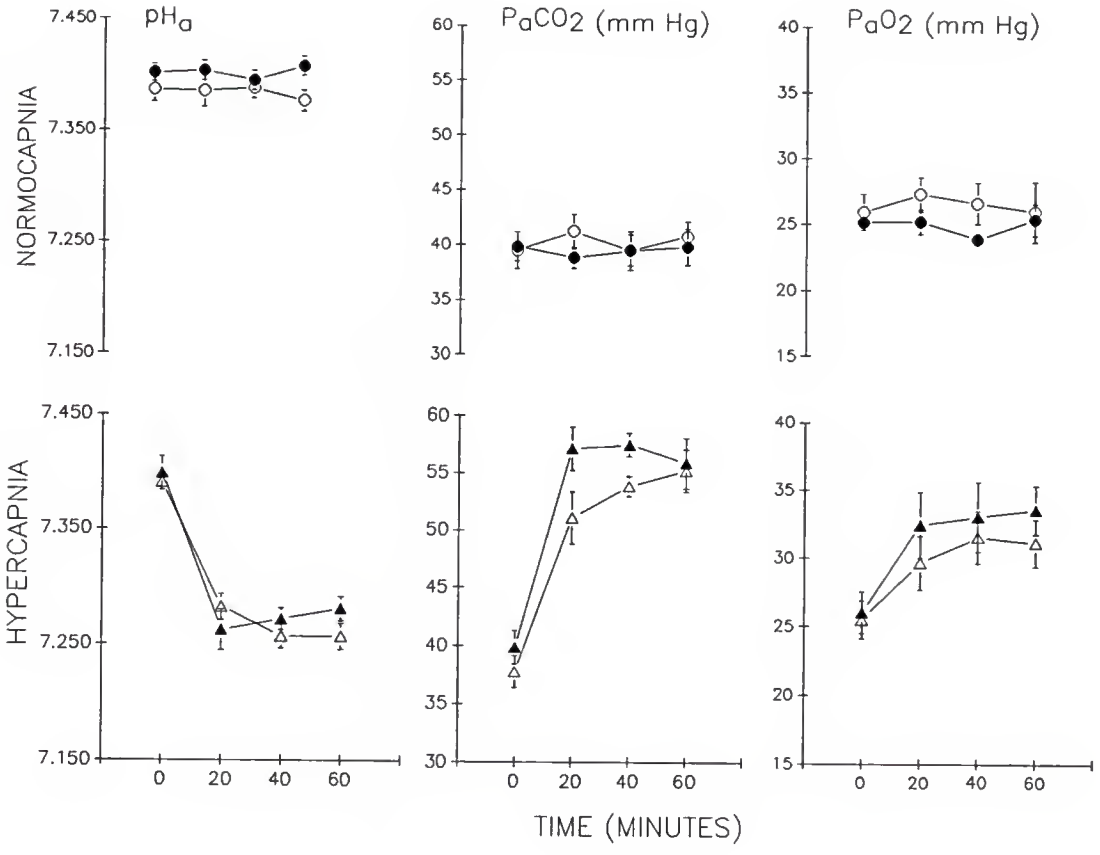

Figure 7–1. Mean fetal pH_a (left), Pa_{CO2} (middle), and Pa_{O2} (right) in normocapnia (upper) and hypercapnia (lower) studies. Open and filled symbols represent intact and denervated fetuses respectively.

Plasma potassium concentration significantly increased during hypercapnia from 3.37 ± 0.07 and 3.42 ± 0.11 to 3.62 ± 0.10 and 3.63 ± 0.19 meq/L in intact and denervated fetuses respectively (Table 7–1).

There was also a small but significant increase in plasma sodium concentration from 142.3 ± 0.8 to 143.7 ± 0.7 meq/L in intact fetuses during hypercapnia (Table 7–1).

HR was significantly increased by hypercapnic acidemia only in intact fetuses (Figure 7–2). During normocapnia, there was a significant overall difference in HR between intact and denervated fetuses, probably reflecting different initial values. CVP was decreased slightly but significantly after 30 min during normocapnia in the denervated fetuses. CVP did not change significantly in the other groups. There seems to be some fluctuations of both MAP and CVP in both intact and denervated fetuses. MAP decreased at 20 and 25 minutes in denervated fetuses compared to the MAP at 0 minutes, and then returned to normal (Figure 7–2).

There were no significant changes in plasma ACTH concentration in either normoxia or hypercapnia experiments (Figure 7–3, upper panel). Plasma cortisol concentration was equally and significantly increased in both intact and denervated fetuses at 40 and 60 minutes (Figure 7–3, lower panel). The increases in plasma cortisol were equal in the intact and denervated groups. The plasma concentration of AVP was approximately doubled during hypercapnia; denervation did not attenuate the AVP response (Figure 7–4).

7.3.2 Responses in Ewes

Hypercapnia significantly decreased maternal pH_a from 7.509 ± 0.008 to 7.353 ± 0.011; Pa_{CO2} increased from 28.4 ± 1.1 to 44.2 ± 0.9 mm Hg and Pa_{O2} from 100.2 ± 2.6 to 119.9 ± 4.2 mm Hg, respectively (Figure 7–5). Plasma potassium concentration also increased during hypercapnia from 3.99 ± 0.10 to 4.27 ± 0.10 meq/L (Table 7–2). Plasma sodium concentration increased slightly during hypercapnia at 20 and 60 minute points compared to the 0 minute

Table 7-1. Means & SEs of fetal HCT, plasma potassium & sodium

	NORMOCAPNIA		HYPERCAPNIA	
HCT (% PCV)	INT	DEN	INT	DEN
0	29.1 ± 3.1	27.0 ± 2.0	27.5 ± 2.1	26.5 ± 0.5
20	29.4 ± 3.2	27.2 ± 2.0	27.8 ± 2.0	26.3 ± 0.6
40	28.8 ± 3.2	27.1 ± 1.9	27.5 ± 2.1	26.0 ± 3.7
60	30.1 ± 3.7	27.1 ± 2.0	27.2 ± 2.0	26.0 ± 0.7
PLASMA POTASSIUM (meq/L)				
0	3.36 ± 0.11	3.72 ± 0.11	3.37 ± 0.07	3.4 ± 0.11
20	3.41 ± 0.11	3.68 ± 0.12	3.51 ± 0.08	3.4 ± 0.14
40	3.41 ± 0.12	3.73 ± 0.10	3.57 ± 0.08*	3.6 ± .19*
60	3.47 ± 0.11	3.77 ± 0.11	3.62 ± 0.10*	3.6 ± .24*
PLASMA SODIUM (meq/L)				
0	144.0 ± 2.5	144.7 ± 0.9	142.3 ± 0.8	144.7 ± 0.5
20	143.2 ± 1.6	145.0 ± 1.1	143.2 ± 0.8*	145.5 ± 0.5*
40	141.9 ± 0.8	144.3 ± 0.8	143.7 ± 0.7*	145.0 ± 0.5
60	141.6 ± 0.5	144.3 ± 0.7	143.2 ± 0.7*	144.6 ± 0.4

* Significant difference between the variables of certain time point and their 0 minute measurements.

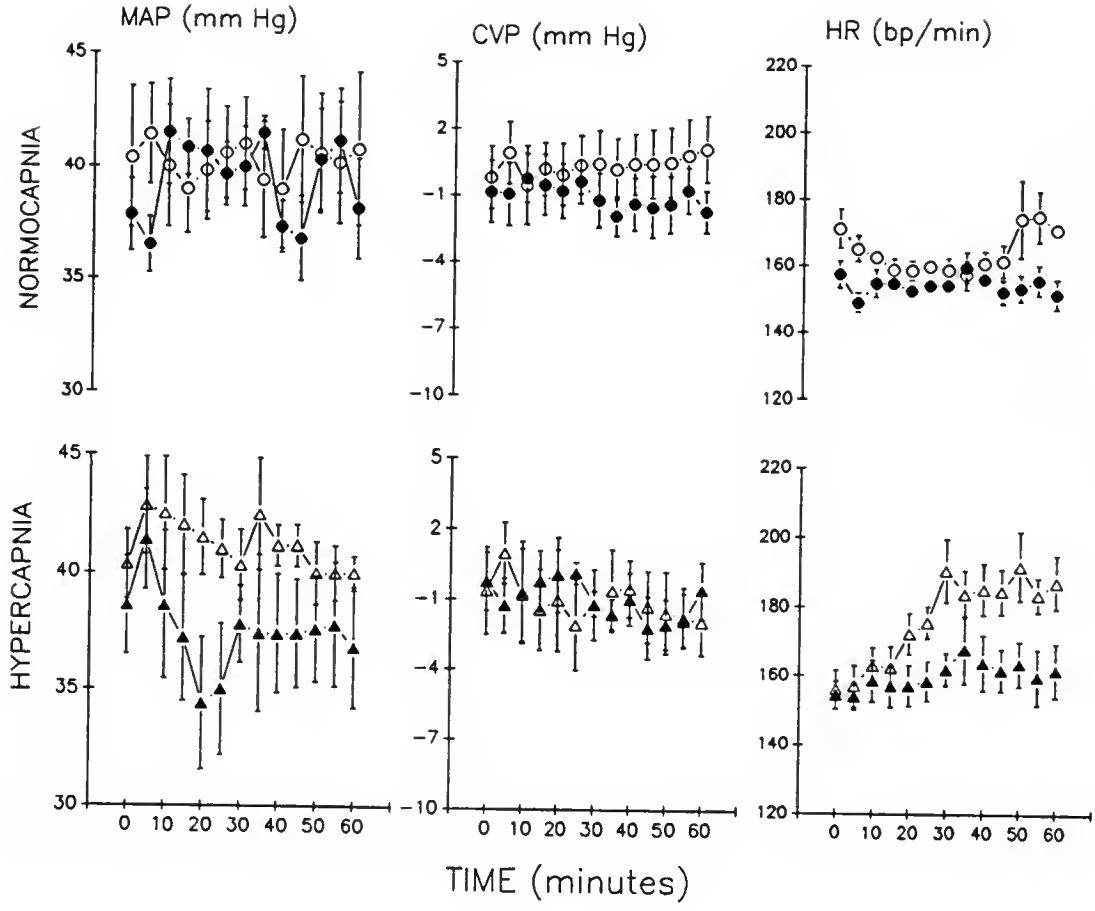

Figure 7-2. MAP (left), CVP (middle) and HR (right) in normocapnia (upper) and hypercapnia (lower) studies. Open and filled symbols represent intact and denervated fetuses respectively.

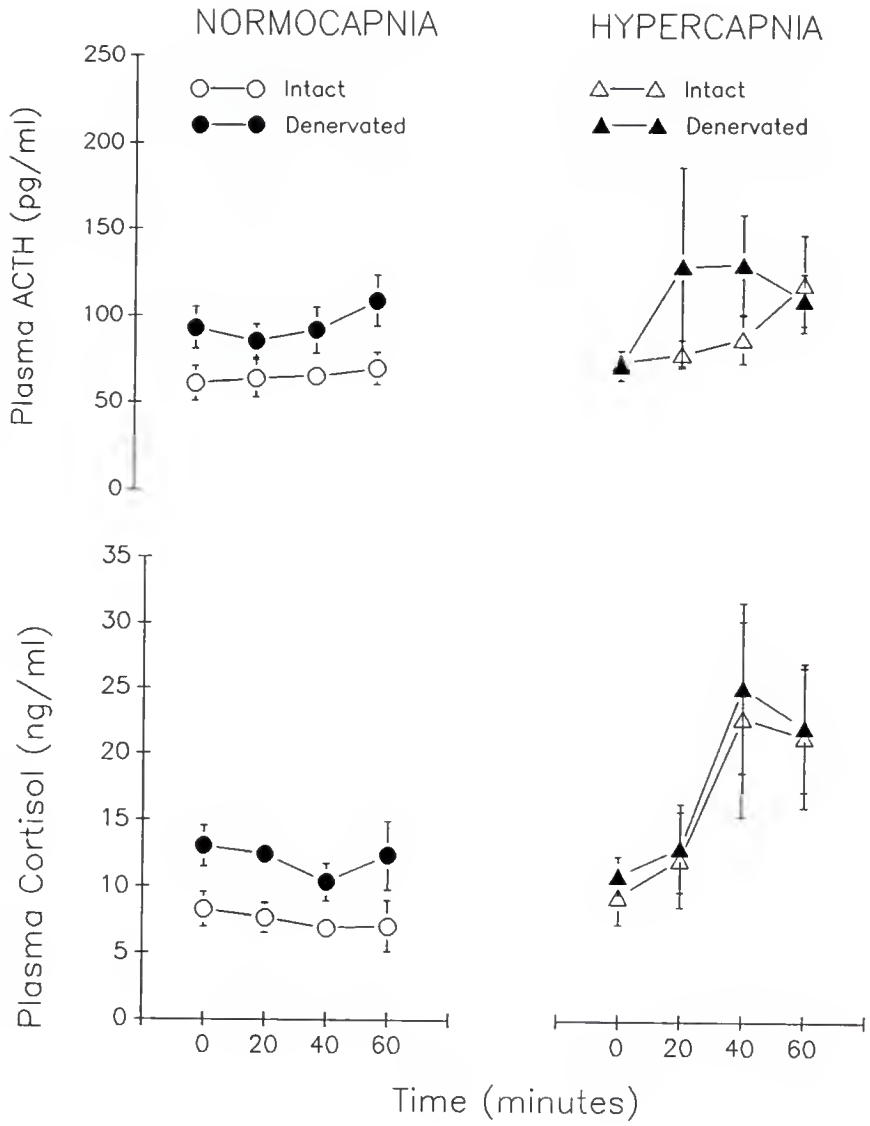

Figure 7-3. Fetal plasma ACTH (upper) and cortisol (lower) concentration in normocapnia (right) and hypercapnia (left) studies. Open and filled symbols represent intact and denervated fetuses respectively.

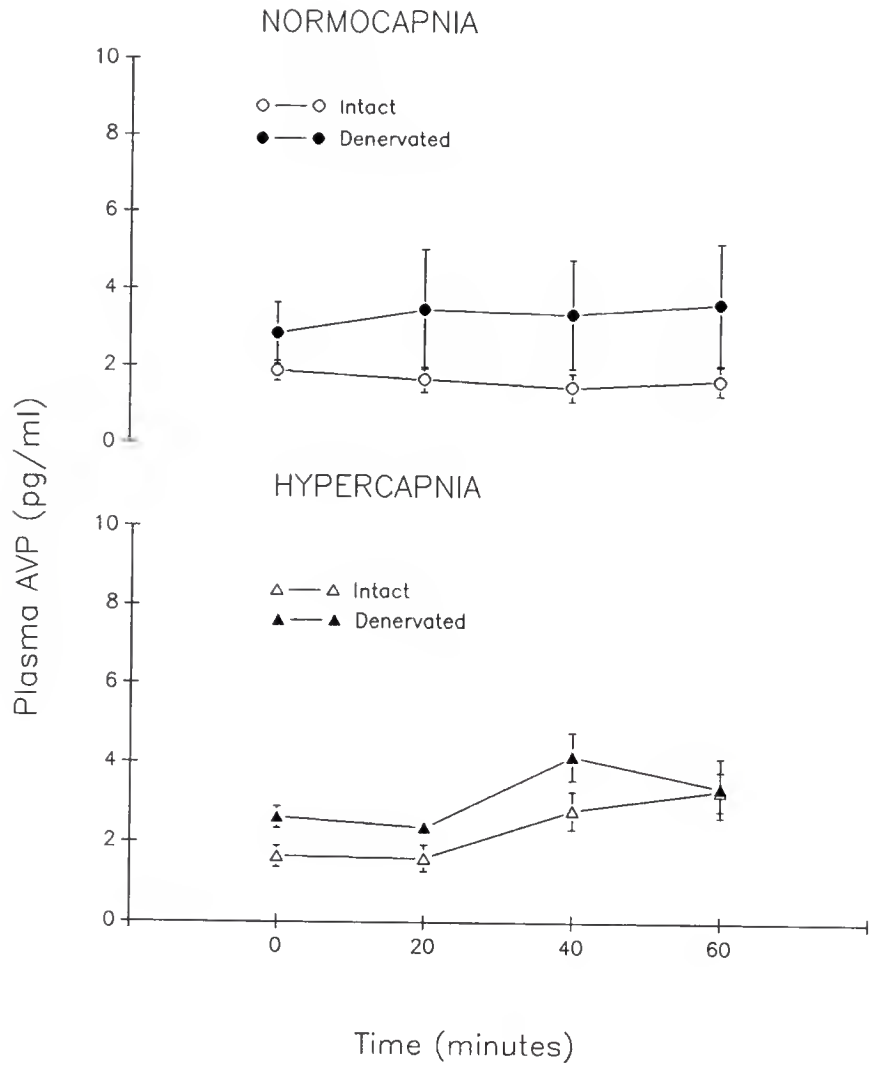

Figure 7–4. Fetal plasma AVP concentration in normocapnia (upper) and hypercapnia (lower) studies. Open symbols represent intact fetuses, filled symbols represent denervated fetuses.

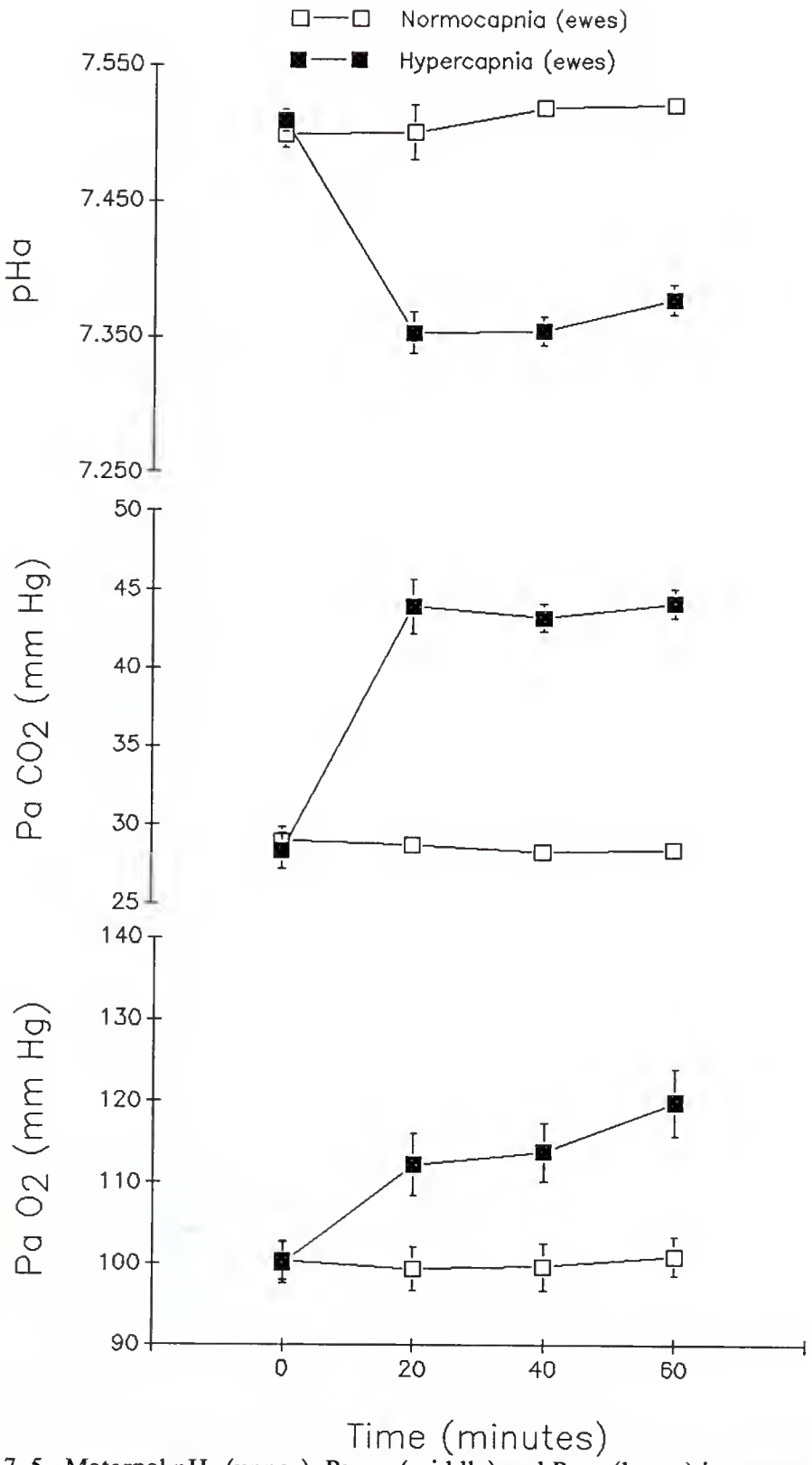

Figure 7–5. Maternal pH_a (upper), Pa_{CO2} (middle) and Pa_{O2} (lower) in normocapnia (open squares) and hypercapnia (filled squares) experiments.

Table 7–2. Maternal means & SEs of HCT, plasma potassium & sodium

	NORMOCAPNIA	HYPERCAPNIA	
HCT (% PCV)			
0	21.8 ± 0.7	22.0 ± 0.9	
20	21.4 ± 0.7	23.8 ± 1.2	* #
40	21.5 ± 0.7	24.3 ± 0.9	* #
60	21.2 ± 0.7	23.7 ± 0.9	* #
PLASMA POTASSIUM (meq/L)			
0	3.93 ± 0.05	3.99 ± 0.10	
20	3.89 ± 0.05	4.17 ± 0.11	* #
40	3.92 ± 0.05	4.20 ± 0.11	* #
60	3.89 ± 0.06	4.27 ± 0.10	* #
PLASMA SODIUM (meq/L)			
0	148.0 ± 0.4	148.8 ± 1.6	
20	146.5 ± 0.8	149.1 ± 0.7	* #
40	147.8 ± 0.5	148.8 ± 0.5	
60	148.0 ± 0.5	149.2 ± 0.3	*

significant difference between groups by Duncan's multiple range test.
* Significant difference between the variables of certain time point and their 0 minute measurements.

variable (Table 7–2). Hct increased slightly during hypercapnia (Table 7–2).

Plasma ACTH and AVP increased significantly at 40 and 60 minutes and plasma cortisol significantly increased at 20 and 40 minutes compared to their 0 minute values (Figure 7–6).

7.4 Discussion

The results from this and other studies in this laboratory (Chapter 4) suggest that decreases in pH_a and/or increases in Pa_{CO2} stimulated fetal ACTH and AVP secretion. In Experiment II (Chapter 5), we intravenously infused HCl into fetuses causing a decrease of pH_a from 7.400 to 7.033 and an increase of Pa_{CO2} from 37.8 to 55.2 mm Hg. In the present study, we produced approximately the same degree of hypercapnia (37.8 to 55.2 mm Hg) but less acidemia (pH_a decreased from 7.390 to 7.257). ACTH was not stimulated and AVP responses to hypercapnic acidemia in the present study were milder than those during infusion of HCl, suggesting that hypercapnia alone is a mild stimulus to AVP and ACTH. Further reductions of pH_a via metabolic acidosis appears greatly increase the strength of the stimulus. This is consistent with the results of a study from the laboratory of Daniel (Daniel et al., 1983), in which AVP was more highly correlated to pH_a than to Pa_{CO2}. The design of the present study does not, however, confirm that H^+ is the only stimulus to AVP secretion during hypercapnia, as suggested by other authors (Wang et al., 1984; Faucher et al., 1987).

Acid infusion experiments in dogs, goats, and rats (Augustinsson and Forslid, 1989; Perez et al., 1979; Welbourne, 1976) suggest that H^+ itself can act as a stimulus to ACTH secretion. In anesthetized dogs, Richards (1957a) dissociated the decrease of pH_a from an increase of Pa_{CO2} by HCl or $NaHCO_3$ infusion, and demonstrated that changes in either H^+ or Pa_{CO2} alone could stimulate the hypothalamus–pituitary–adrenalcortical axis. Results of studies in which adult animals were subjected to ventilatory hypercapnia are not consistent. In some studies, ACTH was increased (Augustinson et al., 1989; Bloom et al., 1977; Raff et

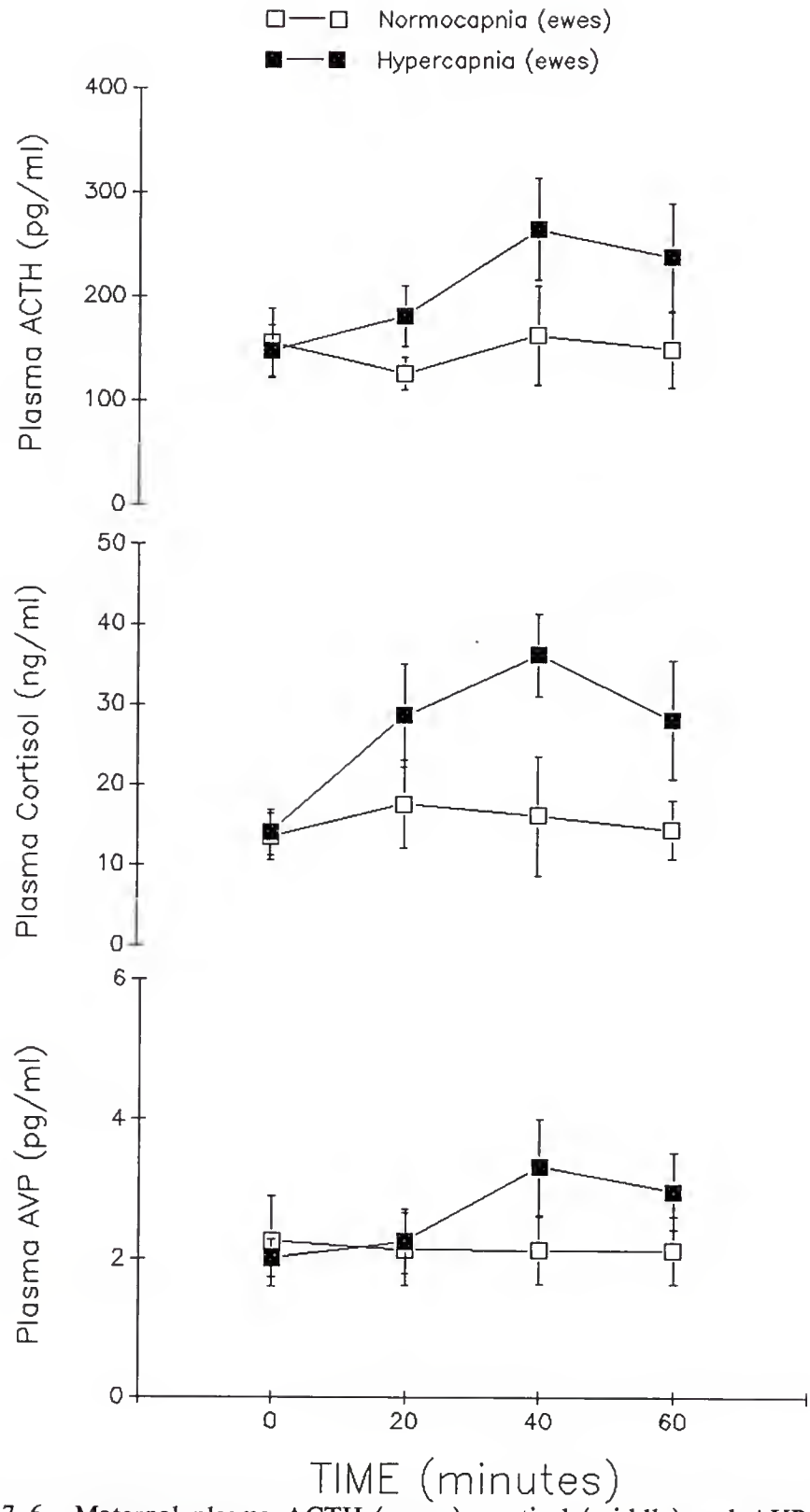

Figure 7-6. Maternal plasma ACTH (upper), cortisol (middle) and AVP (lower) concentration in normocapnia (open squares) and hypercapnia (filled squares) studies.

al., 1988; Richards and Stein, 1957b) while in others ACTH was not changed (Raff et al., 1983; 1984). Hypercapnia augments the ACTH, cortisol and AVP responses to hypoxia in anesthetized dogs and conscious rats (Raff et al.,1983; 1984; 1988).

Plasma cortisol concentration increased to the same magnitude in both intact and denervated fetuses in the absence of increased plasma ACTH. The increase in fetal cortisol was approximately 20 minutes later than the increase in maternal cortisol, and most likely reflects the transplacental transfer of cortisol from the maternal to the fetal circulation as suggested by a previous study (Wood, 1987).

We conclude that both ACTH and AVP responses to slow hemorrhage are not mediated by peripheral chemoreceptors and that AVP responses to slow hemorrhage might be partially mediated by central chemoreceptors.

CHAPTER 8
SUMMARY

Hemorrhage has long been used as an effective stimulus to test cardiovascular or hormone responses because it is an easily controlled and quantified stressor. Discovering the regulatory mechanisms for hormonal responses to hemorrhage is important, because hemorrhage is a common factor that might affect plasma hormone levels during animal research whenever blood sampling is required. It is especially important in fetal studies because a large amount of blood is required for measuring hormone levels, compared to the fetal total blood volume.

The main purpose of the study was to investigate the afferent pathway for regulating ACTH, cortisol, AVP and renin responses to slow hemorrhage in the chronically prepared, late gestational fetal sheep. It is known that in adult animals, atrial type-B receptors play an important role in regulating these hormonal responses to mild or non-hypotensive hemorrhage. Not much was known about fetuses in this area. This study offered a step by step investigation to systematically examine the role of various components of cardiovascular reflex control of fetal hormone secretion during slow hemorrhage. A total of forty-six fetal sheep at 121 to 140 days gestation, and four series of experiments were covered in this dissertation.

In the first series of experiments, we hypothesized that the afferent pathway is the same in fetuses as in the adults. Eleven fetal sheep were studied. Five of the fetuses were vagotomized to interfere with the afferent pathway from atrial type B receptors, and six remained intact as controls. Four to five days later, we hemorrhaged the fetuses with a rate of 11 ml every 10 min for a total of 120 minutes. We found that there were no significant

differences in the hormonal responses between groups. This result led to a conclusion that the afferent pathway for regulating mild or non-hypotensive hemorrhage is different in fetuses than in the adults. Fetal hormonal control during hemorrhage is not primarily mediated by the atrial type B receptor. Experiment I also suggested that the hormonal responses to hemorrhage in the fetuses might be stimulated by chemoreceptor activity, triggered by the acidemia or hypercapnia of fetal hemorrhage, since further analysis reveals that the hormonal responses showed a stronger correlation to arterial pH than to arterial blood pressures.

The second series of experiments was designed to test the hypothesis that ACTH, AVP and PRA are stimulated by chemoreceptors in late gestational fetal sheep. We intravenously infused HCl into fetuses, which resulted in a large decrease in pH_a, and an increase of Pa_{CO2}. Plasma ACTH, cortisol and AVP concentrations were significantly increased but PRA was not statistically changed. We speculated that the lack of variation in PRA might be due to the inhibitory effect of Cl^-. This study showed that metabolic acidemia does stimulate ACTH, cortisol and AVP secretion.

We designed the third series of experiments to test the peripheral chemoreceptors' effect during hemorrhage. We compared the hormonal responses between intact and carotid denervated plus vagotomized fetuses, with or without phenylephrine infusion. Phenylephrine was used to control blood pressure and to maintain placental flow, to avoid the more severe changes of blood pressure and hypercapnic acidemia in denervated fetuses compared to intact ones. Hemorrhage stimulated secretion of all four hormones in both intact and denervated fetuses. The fact that peripheral chemoreceptor denervation did not attenuate the hormonal responses disproved our hypothesis that peripheral chemoreceptors mediate the responses. Phenylephrine infusion into denervated fetuses successfully maintained the acid-base balance at intact fetal levels, caused attenuation of ACTH and cortisol, and inhibited AVP release. This suggested a possible central chemoreceptor's effect on ACTH and AVP. There was no

effect of denervation with or without infusion of phenylephrine on PRA during hemorrhage, the data indicate that chemoreceptor is not important for fetal PRA responses to slow hemorrhage.

Since the above experiments suggested that the stimulation of fetal ACTH, cortisol and AVP might be related to the degree of hypercapnic acidemia; i.e., there might be a threshold for initiating the responses, we further investigated the peripheral chemoreceptor's effect in the fourth series of experiments. A lesser degree of hypercapnic acidemia, which is closer to the changes that occured during hemorrhage than to the large decline of pH_a during HCl infusion, was induced by letting ewes breathe hypercapnic mixed gases. The fetuses were divided into two groups: intact or carotid sinus denervated plus vagotomized fetuses. Each fetus participated in both the normocapnia control and hypercapnia experiments. We found that hypercapnia, associated with small decreases in pH_a, did not stimulate ACTH in either intact or denervated fetuses. AVP was mildly stimulated, and there were no differences between intact vs denervated fetuses. The conclusion drawn from this experiment is: the stimulation of ACTH during hemorrhage is not mediated by peripheral or central chemoreceptors, and AVP is not mediated by peripheral, but possibly by central chemoreceptors.

From the above experiments, we can conclude that the afferent pathway for mediating ACTH, AVP and PRA responses to slow hemorrhage in fetuses is different from that in adults. The fetal hormonal responses are not regulated by afferent fibers located in cervical vagosympathetic trunks or in carotid sinus nerves, are not related to changes of mean arterial and central venous pressures, and are not related to changes in pH_a and Pa_{CO2} in peripheral blood. Only AVP might be partly regulated by central chemoreceptors.

The question still needs to be answered: what is the possible afferent pathway in mediating fetal ACTH, AVP and PRA responses to slow hemorrhage?

First, we start by examining the methods used for testing peripheral mechano– or chemoreceptors' effects, one of which is to denervate the nerve fibers and compare hormonal responses between intact and denervated fetuses. Denervation successfully deafferents the non–sympathetic afferent fibers from the carotid sinus, aortic arch, and the heart and lungs, but whether there are afferent fibers within the sympathetic nerves via the heart still remains intact. We know that the sympathetic nervous system is functional by the age of the fetal lambs that were studied. Thus, we cannot discount that the afferent signal is transmitted by the remaining sympathetic fibers.

Signals other than the mechano– or chemoreceptors mentioned in the study might affect hormone secretion. One of the disadvantages of using hemorrhage as a stimulus is that it is very difficult to avoid the extensive secondary responses of the body. Various compensatory mechanisms can occur simultaneously, and have an effect on each other, which makes it difficult to identify the specific regulatory factor. For example, decreased plasma osmolality can have a negative effect on AVP secretion, since fetuses have a greater ability to defend their blood volume from hemorrhage. The dilution of the blood by plasma volume restitution from other fetal water compartments might cause a decrease in plasma osmolality. It appears that this negative effect of osmolality on AVP secretion could be overridden by other stimulation factors during hemorrhage on AVP. Also, AVP's central effect is to stimulate ACTH secretion, while the increase of Angiotensin II will inhibit renin production.

Hemorrhage can stimulate production of other vasoactive substances, such as catecholamines, serotonins, prostaglandins, etc., which might affect the system either directly or indirectly. The possible influence of changes in local homeostasis of the endocrine or metabolism during hemorrhage might influence the secretion or degradation rate of the hormones. Also, there might be more direct central stimulation of the hormone secretion for ACTH and AVP in fetuses by blood borne substances because of the immaturity of the fetal blood brain barrier.

Another consideration is analysis of plasma hormone concentration, i.e., the hormone from other sources. Experiment IV suggested a transplacental movement of cortisol from the maternal side (Chapter 7). Studies from other laboratories suggest a placental origin for ACTH and CRF. We cannot identify the sources of plasma hormone from the current experiments. But it is clear that to reveal the mechanism of the placenta hormones regulation during hemorrhage will help us to better understand the endocrine control mechanism during fetal life.

Conclusions:

1. Slow hemorrhage stimulates ACTH, cortisol, AVP and PRA in late gestational fetal sheep, which confirmed previous findings by others.
2. The atrial type B receptor does not regulate fetal response, which suggests that the afferent pathway for regulating fetal hormonal responses to hemorrhage is different from that in adults.
3. Chemoreceptors are active during fetal life, and are involved in mediating fetal ACTH, cortisol and AVP responses to severe metabolic acidemia caused by HCl infusion. The hormonal responses seem to be more strongly with pH_a than with Pa_{CO2} changes.
4. Peripheral chemoreceptors are not responsible for the ACTH, cortisol and AVP secretion during a milder degree of hypercapnic acidemia, which simulates to the plasma acid-base situation during slow hemorrhage. Central chemoreceptors are thought to be partly involved in mediating AVP but not ACTH secretion.
5. Thus, from points 3 and 4, there might be a threshold for initiating the chemoreceptor mediated hormonal responses. AVP appears to be more sensitive than ACTH to stimulation by hypercapnic acidemia.
6. Finally, the afferent pathway for regulating fetal hormonal responses to slow hemorrhage remains unknown. Central chemoreceptors might be mildly involved in

the responses. The effect from the cardiac sympathetic afferent fibers needs to be further investigated.

7. The influence of carotid sinus baroreceptors and/or chemoreceptors is important for the maintenance of blood pressure and blood gases during hemorrhage.

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BIOGRAPHICAL SKETCH

Hong-gen Chen was born in Haikou city, Hainan province, China. She graduated from the First Medical College in Gaungzhou in February 1977, finished her internship in 1978, and worked as a resident of pediatrics until she received another three-year advanced medical training program from February 1982 to December 1984 in the Third Medical College in Chongqing. Since then, she worked in the Pediatric Department of Nanfang Hospital in Guangzhou. She came to the United States and worked in Dr. Willa Drummond's laboratory as a visiting scholar for about nine months. She was admitted as a graduate student of the University of Florida, College of Medicine, Department of Physiology in Fall, 1986, and has been working toward her Ph.D. degree with a major in Perinatal Physiology under the guidance of Dr. Charles E. Wood. She plans to graduate in the Spring of 1991.

She married her husband Ke Wu in 1977, and is going to happily celebrate their fourteen year wedding anniversary in California at the end of this year.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Charles E. Wood, Chairman
Associate Professor of Physiology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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This dissertation was submitted to the Graduate Faculty of the College of Medicine and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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